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TOWNSEND and TOWNSEND and CREW LLP

By: Jo Ann Honcik Dallara

PATENT
Attorney Docket No. 019904-002210US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Joseph K. Belanoff

Application No.: 10/519,008

Filed: December 21, 2004

For: METHODS FOR TREATING PSYCHOSIS ASSOCIATED WITH INTERFERON-ALPHA THERAPY

Confirmation No. 7228

Examiner: Brooks, Kristie Latrice

Technology Center/Art Unit: 1616

APPELLANT'S BRIEF UNDER 37 CFR §41.37

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Further to the Notice of Appeal mailed on June 5, 2009, for the above-referenced application, Appellant submits this Brief on Appeal.

A Petition for a three-month extension until November 5, 2009, accompanies this communication. Appellant authorizes the Commissioner to deduct the requisite Small Entity fee for the Petition of $555.00 and of $270.00 for filing the Brief, should additional fees be owed, the Commissioner is authorized to deduct such a fee from the undersigned's Deposit Account No. 20-1430.

TABLE OF CONTENTS

1. REAL PARTY IN INTEREST

The real party in interest is the assignee, Corcept Therapeutics, Inc., of Menlo Park, California.

2. RELATED APPEALS AND INTERFERENCES

This appeal has no related proceedings or interferences.

3. STATUS OF CLAIMS

A. TOTAL NUMBER OF CLAIMS IN APPLICATION

The claims in the application are 1-22

B. STATUS OF ALL THE CLAIMS IN THE APPLICATION

Claims canceled: 20-22

Claims withdrawn from consideration but not canceled: 5-7

Claims pending: 1-4 and 8-19

Claims allowed: None

Claims rejected: 1-4 and 8-19

C. CLAIMS ON APPEAL

The claims on appeal are 1-4 and 8-19.

4. STATUS OF AMENDMENTS

An amendment after Final Rejection was not filed. Therefore, claims 1-4 and 8-19 on appeal are as amended in the Response to the Office Action filed on August 8, 2008.

5. SUMMARY OF CLAIMED SUBJECT MATTER

The pending claims are not separately appealed. The subject matter of claim 1 is directed to a method of treating psychosis induced by long-term use of interferon alpha [IFN-α]. IFN-α is a drug used to treat a variety of chronic disorders and psychosis is a known side effect of long-term treatment. The invention is the use of an anti-glucocorticoid receptor antagonist to protect the brain from psychotic episodes induced by alpha-interferon.

CLAIM 1-INDEPENDENT

Claim 1 finds support in the specification as follows:

The preamble: A method of ameliorating the symptoms of psychosis associated with interferon α therapy in a patient, comprising: *In the specification at page 2, paragraph 5 and original claim 1.*

Step: administering to the patient having received interferon α therapy and suffering from psychosis associated with the interferon α therapy, an amount of a glucocorticoid receptor antagonist effective to ameliorate the symptoms of psychosis in the patient, *In the specification at page 2, paragraph 5 and original claim 1.*

Proviso: with the proviso that the patient is not otherwise in need of treatment with a glucocorticoid receptor antagonist. I*n the specification at page 2, paragraph 5, and original claim 1.*

TECHNICAL OVERVIEW

Psychosis is a psychiatric condition defined by hallucinations and delusional thinking. Hallucinations can be visual, tactile or auditory. Like a fever from a cold, sepsis or flu, psychosis is a symptom of a variety of diseases or syndromes. When a syndrome creates a glucocorticoid dysregulation, the resulting psychosis is treatable with a glucocorticoid receptor antagonist [GRA].

Some background is helpful. The primary glucocorticoid in humans is called cortisol. It is also called the 'stress' hormone because its level in the body increases as we experience stress. Cortisol binds to the glucocorticoid receptor [GR]. GRAs inhibit the activity of cortisol by blocking its binding to the GR.

The connection between some types of psychosis and excess glucocorticoid is part of the prior art. Not all psychotic events are treatable with GRAs. In particular, the psychotic symptom of schizophrenia and schizoaffective disorder are not treatable with GRAs. The present invention is the discovery that GRA can ameliorate psychotic episodes associated with long-term use of alpha interferon [INF-α].

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There is a single rejection under 35 U.S.C. §103. The Examiner rejects the pending claims 1-4 and 8-17 as obvious over the inventor's earlier work, Schatzberg and Belanoff (US 6,1560,349) in view of Ademmer *et al.*, "Suicidal Ideation with IFN-α and Ribavirin in a Patient with Hepatitis C," **Psychosomatics** 42-4, 365-367, and Shimizu *et al*, "Increase In Serum Interleukin-6, Plasma ACTH And Cortisol Levels After Systemic Interferon-α Administration," **Endocrine Journal** 42(4):551-6, 1995 (abstract).

Claims 18-19, which depend from claim 1 and recite patients with specific diseases and substance abuse respectively, are further rejected as obvious in view of Dieterich "Treatment of Hepatitis C and Anemia in Human Immunodeficiency Virus-Infected Patients," **The Journal of Infectious Diseases** 185 (Supple 2:S128-37, 2002). Dietrich is cited for disclosing neuropsychiatric side effects of long-term use of IFN-α in patients with the diseases recited in claim 18 and addictions of claim 19.

7. ARGUMENT

The subject appeal presents a straightforward case of an appellant rebutting a *prima facie* case by presentation of a Rule 132 Declaration with Exhibits 1-7 (Exhibits A-G). The Examiner responds that the evidence is not persuasive.

The Board's attention is drawn to a minor inconsistency in the prosecution history. The Examiner officially presents her *prima facie* case of obviousness based on two references, Schatzberg and Ademmer. However, in response to appellant's arguments to a non-final Office Action, she added Shimizu as a secondary reference to re-establish her *prima facie* case of obviousness. To expedite prosecution, appellant waived the right to request a new, non-final Office Action and responded with a Rule 132 Declaration (Exhibit A). The declaration was

entered by the Examiner on March 10, 2009. All these actions were within the discretion of the Examiner and the application is properly before this Board.

In the appellant's opinion, the Examiner's reasoning for maintaining the obviousness was unclear as presented in the various Office Actions, especially in view of the reference teaching away (Gisslinger - Exhibit D). At the appellant's request, the Examiner kindly presented an extensive argument on August 21, 2009, in a second Advisory Action. Appellant is grateful for the detailed explanation and will focus the remaining comments on the reasoning set forth in that Advisory Action.

There appears to be no misunderstanding of the position of the appellant in his Rule 132 Declaration nor of the 6 references presented as exhibits. This Board is presented with a factual question of adequacy: In view of the claim language, is the evidence when viewed as a whole adequate to rebut the *prima facie* case of obviousness. The Board is already aware of the legal standards applicable to such circumstances and appellant would only remind the Board of Judge Plager in his concurrence in *In re Oetiker*, 24 USPQ 2d 1443 at 1447 (Fed. Cir. 1992):

> Specifically, when obviousness is at issue, the examiner has the burden of persuasion and therefore the initial burden of production. Satisfying the initial burden of production and thus initially the burden of persuasion, constitutes the so-called prima facie showing. Once that burden is met, the applicant has the burden of production to demonstrate that the examiner's preliminary determination is incorrect. ... **If, as a matter of law, the issue is in equipoise, the applicant is entitled to the patent. [emphasis added]**

Rejected claim 1 plainly reads on a method of ameliorating the symptoms of psychosis associated with interferon α therapy in patients taking INF-α and suffering from psychosis.

The Examiner initially presented her *prima facie* case of obviousness by combining Schatzberg (appellant's own work) disclosing the treatment of psychosis associated with glucocorticoid dysregulation with GRAs, with Ademmer teaching neuropsychiatric problems in a single patient taking IFN-α (see Office Action mailed May 7, 2008, @ page 4). When faced with appellant's argument that neither Schatzberg nor Ademmer suggested that IFN-α associated psychosis was related to glucocorticoid (cortisol) dysregulation, the Examiner

completed her *prima facie* case of obviousness with Shimizu. Shimizu disclosed an increase in serum cortisol after injection with IFN-α.

Appellant was unaware of the Shimizu reference and was surprised to learn of an elevation in cortisol from treatment with IFN-α. However, further investigation determined that the phenomenon observed by Shimizu was taken out of the context of the totality of the relevant prior art. According to the literature, IFN-α temporarily causes the body to experience stress by inducing a fever. The elevation is transitory and cortisol levels return to normal a few hours after administration of IFN-α.

Gisslinger (Exhibit D) expressly teaches that this elevation of cortisol is not only transitory but temporary. Over several weeks, the patient adapts to the drug and cortisol levels no longer increase upon administration of IFN- α and remain at normal levels thereafter. Importantly, INF-α associated psychosis does not occur until there have been at least **three months** of continued use with IFN-α. Appellant provided a Rule 132 Declaration with academic references backing up each of these relevant facts (Exhibits A-G).

The medical facts are not in dispute. Neither is the Examiner's adherence to the give and take of a *prima facie* case of obviousness. The Examiner **has not** ignored the evidence of teaching away. Her position is articulated in the Advisory Action mailed on August 21, 2009.

The Board is asked to review the **strength** of her position relative to the references and the rejected claims. For it is the Examiner's burden to present a compelling argument that rebuts the totality of the evidence and re-establishes the propriety of her *prima facie* case of obviousness.

Appellant urges that the Examiner's arguments are not adequate to rebut the *prima facie* case of obviousness because they rely on unreasonable interpretations of the claims, ignore claim limitations, are circular, and in some instances, are not relevant to the argument at hand.

In the first paragraph on page 2 of the Advisory Action, the Examiner argues that the fact that cortisol levels drop well before the onset of psychosis is not deemed convincing because

> Applicant is not claiming the treatment of a disease, but ameliorating (e.g. to improve) the symptoms of psychosis associated with IFN-α therapy.

In logic, the above argument is known as an "irrelevant truth." It is true that appellant is claiming the treatment of a symptom, not a disease. It is just like using aspirin to treat the fever of a cold, but not to treat the cold virus *per se*. To properly set forth her *prima facie* case of obviousness in view of the totality of evidence, the Examiner must argue that the prior art suggests the use of a GRA to treat IFN-α associated psychosis despite the fact that the psychosis and elevated cortisol happen months apart. The fact that the claim recites ameliorating a symptom is simply an irrelevant truth.

In the first paragraph on page 2, the Examiner next argues:

> Applicant does not have any claim drawn to cortisol, any level in which cortisol is to be present, any time frame in which the glucocorticoid receptor antagonist is to be administered (e.g. during or after IFN-α therapy), or any specific amount of (or dosing regimen) of IFN-α that is to be administered to the patient.

The three arguments set forth above are more irrelevant truths. They simply do not further the Examiner's position that the prior art suggests the use of GRAs for treating IFN-α associated psychosis.

1. **Cortisol Levels** – The Examiner is presumably urging that the rejected claims fail to state that cortisol levels are normal when pyschosis arises and GRA therapy begins. This is irrelevant because to the best knowledge of the relevant literature (Gisslinger), cortisol levels are normal when treatment begins. And if for some unknown reason, the patient does exhibit high levels of cortisol, the proviso language would preclude the claim scope from unintentionally overreaching to those patients. The proviso language reads:

> with the proviso that the patient is not otherwise in need of treatment with a glucocorticoid receptor antagonist.

The proviso language was intended to preclude the claim from reading on those rare patients who perhaps have both psychotic major depression (Schatzberg '249) and some disease amenable to IFN-α therapy. And, this language also precludes the claim scope from

encompassing those patients taking IFN-α, exhibiting elevated cortisol for unknown reasons and having psychosis.

2. **Time Frame** - The Examiner presumably urges that the claim could read on patients no longer taking IFN-α. Again this argument is irrelevant to the issue at hand because it does nothing to support the position that the prior art suggests the use of GRAs to treat IFN-α associated psychosis. Moreover the plain language of the claim states that:

> administering to the patient having received interferon α therapy and suffering from psychosis associated with the interferon α therapy,

Apparently the Examiner reads the claim to embrace persons who took IFN-α, stopped taking IFN-α, and later developed psychosis. Clearly, the Examiner has stretched her obligation to interpret the claims as broadly as is reasonable to the realm of unreasonable.

If a patient is no longer taking IFN-α, the psychosis is not "associated with the interferon α therapy." The specification was carefully crafted to avoid inadvertent overreaching. For example, on page 9, ¶30, the section on diagnosis of patients with psychosis associated with IFN-α are clearly defined as those taking the drug and typically not having a prior history of psychosis. As the specification expressly states on page 4, ¶ 13:

> Psychosis associated with "interferon-α therapy" refers to a psychosis that is induced by interferon- α therapy and is not associated with depression. Thus, "psychosis associated with interferon-α therapy" includes psychotic disorders associated with interferon-α treatment, but not psychotic disorders associated with depression, as in for example, psychotic major depression.

IFN-α therapy is a long-term therapy for chronic conditions. If psychosis occurs, the IFN-α is terminated (see Specification, page 1, ¶3 @ lines 26-27). The problem is that the chronic condition that IFN-α was holding in check is still present. The appellant's invention permits the important benefits of IFN-α therapy to continue. The specification has an entire section on page 29, ¶ 85-86, describing concomitant administration of the GRAs and IFN-α.

To read the rejected claim as including patients no longer taking IFN-α is contrary to the plain meaning of the claim language, contrary to the teachings of the specification, and contrary to the science underlying the invention. It is clearly an unreasonable interpretation.

3. Dosing – The Examiner complains that the rejected claim 1 does not recite dosing. The rejected claim recites conventional language typical of method of treating claims:

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an amount of a glucocorticoid receptor antagonist
effective to ameliorate the symptoms of psychosis in the patient
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In the first instance, the Examiner's comments in no way support her *prima facie* case of obviousness because reciting amounts will not further the argument that the prior art suggests the use of GRAs to treat IFN-α associated psychosis. Nor does the Examiner attempt to explain why the failure to recite dose or timing of dose is relevant to the obviousness rejection.

In the remaining argument of the first paragraph of the Advisory Action, the Examiner restates her *prima facie* case of obviousness and teachings of Shatzberg, Ademmner, and Shimizu. There is no attempt to link the above three arguments to the *prima facie* case of obviousness based on the trilogy of references. The conclusion of obviousness is a naked conclusion.

In the second paragraph of the Advisory Action, the Examiner argues that the term, "psychosis" is defined as including all psychotic conditions, and because Schatzberg similarly defines psychosis in combination with Ademmer, the pending claims are obvious because one of skill can *assume* that mifepristone (GRA) can be used to treat symptoms associated with IFN-α therapy. Assuming is another word for expectation, and this paragraph argues that Schatzberg and Ademmer, **without** Shimizu (transient cortisol elevation), would set forth a proper *prima facie* case of obviousness. The failure of the combination of Ademmer and Schatzberg to properly set forth a *prima facie* case of obviousness because it lacked evidence of a connection between cortisol dysregulation and IFN- α associated psychosis was argued (Response mailed 8-22-08). In response, the Examiner cited Shimizu in her final Office Action on page 12 (mailed on 12-10-08); but, Shimizu was never formally cited as a primary or secondary reference. For purposes of this appeal, Shimizu is considered to be a secondary reference.

Having set forth a *prima facie* case of obviousness, the appellant responded with a Rule 132 Declaration with evidence rebutting the supporting evidence. Although filed after a final Office Action, the Declaration was properly entered in the first Advisory Action mailed on March 10, 2009.

In the next paragraph, the Examiner urges that the references relied upon by appellant in his Rule 132 Declaration are not persuasive. She begins by again raising the fact that the claims do not recite any levels of cortisol. This argument has been rebutted above and those comments apply here. The failure of the claim to recite normal levels of cortisol is not relevant to the legal question at hand. The Examiner continues by noting that Roosth (Exhibit B) reports that cortisol increases are transient regardless of the amount of units of IFN-α administered. Contrary to the Examiner's position, the fact that cortisol levels increase as the dose of IFN-α increased is irrelevant to the fact that the increasing cortisol levels are a temporary phenomena with cortisol levels returning to normal as the patient adapts to lINF-α therapy.

The Roosth, Muller (Exhibit C) and Gisslinger references were not provided to individually rebut the *prima facie* case of obviousness. The Examiner is correct that Roosth does not teach away from the invention. Neither does Shimizu. But Gisslinger does. The multiple references were presented to place Shimizu in its proper perspective. Historically, Roosth was the first to connect IFN-α with increased cortisol levels and attributed the increase to fever (pyrogenic effects of interferon – page 312). As Dr. Belanoff explains in ¶6 of his declaration, Muller also looked at the mechanism of action for the increased cortisol and noted fever as the likely cause. As with the other prior work, the effect is noted as transient.

Now the Examiner is 100% correct when she notes that from these three earlier references it is obvious that each time you take INF-α, you might expect a fever and increased cortisol levels. Those facts set up the Examiner's side of the *prima facie* case of obviousness.

The Examiner is also correct, that Roosth and Muller do not teach away from the claimed invention. Gisslinger is the reference that teaches away because it alone deals with the effect of **long-term** IFN-α therapy!

The information missing from the first three references is the effect of long-term IFN-α therapy on cortisol levels. Cortisol is the primary glucocorticoid in the human body and is

prevented from binding to the glucocorticoid receptor by the GRAs (glucocorticoid dysregulation). As the Examiner says repeatedly, the first three papers dealt with "the short term effects of IFN-α" therapy. This is correct. It is Gisslinger that evaluated long-term administration of IFN-α.

Gisslinger looks at eight patients with leukemia taking IFN-α therapy. IFN-α doses were at 5 million units administered 5 times per week for three weeks. After three weeks, the patients adapted to IFN-α and their cortisol levels returned to normal. On page 492 is an array of figures demonstrating that the patients adapt to the IFN-α after 3 weeks. Cortisol levels return to normal. ACTH returns to normal. The fever reaction is gone (which induces cortisol production). Here are some quotes from Gisslinger:

> After 3 weeks of INF-α therapy, no significant stimulation of the HPA axis occurred after administration of IFN-α. [abstract]
>
> When the patients were studied again after they had been on IFN-α for 3 weeks, only slightly but not significantly elevated ACTH and cortisol plasma levels compared to baseline day could be observed. [Page 492, 2nd col]
>
> Body temperature increased after the first injection...and returned to baseline level after three weeks of IFN-α treatment. [Page 492, 2nd col]

Although not relied upon by the Examiner, Gisslinger does make the following statement:

> The chronic activation of the HPA axis by IFN-α is reminiscent of the alterations seen in anorexia nervosa, depression, alcoholism, and compulsive running, and could also be responsible for the neuropsychiatric disorders previously described in patients receiving treatment with IFN-α.

According to Gisslinger, the chronic activation of the HPA axis apparently does not include significant increases in cortisol levels. The HPA axis is a complicated multidimensional biological process. So why would one of skill expect a GRA, the function of which is to block cortisol from binding glucocorticoid receptors, to be useful to treat psychotic episodes associated with IFN-α therapy when cortisol levels have returned to normal after three weeks of the start of IFN-α therapy and the psychosis arises months later?

On page 3 of the Advisory Action, the Examiner argues that "it is *possible* that the behavioral changes arise as a result of elevated cortisol levels following administration of IFN-

α." 'Possible' is not the test for a *prima facie* case of obviousness. The prior art has to suggest or motivate one of skill to *expect* the result. The occurrence of any event is *possible* given an infinite amount of time.

Next, the Examiner argues that Gisslinger is not an adequate teaching away because they report a "slightly elevated" level of cortisol (omitting Gisslinger's characterization of the increase as not significant) and because Roosth *et al.* taught that increased doses led to increased cortisol even over 7 weeks, while Gisslinger used a clinically approved regimen of a constant 5 million units 5 times a week for three weeks. Roosth is an early paper (1986) paper reporting on an experimental use of INF-α. In Roosth, the authors are administering single doses of IFN-α one week apart. The weekly doses increased from 1 million units to a huge 120 million units in a single dose! Not surprisingly, there was no observation of adaption to IFN-α under such circumstances.

The reason the Shimizu paper caught Dr. Belanoff by surprise is that in the practical world of clinical use, IFN-α is typically given to patients at less than 10 million units per dose several times a week. Under these real world circumstances, the literature tracks Gisslinger and does not report elevated cortisol levels in patients taking long-term IFN-α for chronic conditions.

The Examiner complains that the claims fail to recite a specific regimen of IFN-α, apparently rationalizing that unless the claim recites an IFN-α dosing protocol that leads to cortisol adaption, the invention is obvious. Although appellant was unaware of the possibility that non-clinically approved use of INF-α in excess of that commonly used in clinic could lead to elevated cortisol levels, the proviso language expressly excludes that situation from the claim. Put more simply, if you are stressing a person with excessive toxic amounts of IFN-α, the *expected* elevation of cortisol leading to psychosis would be excluded by the claims' proviso language.

Let's place the claims back in the real world of clinical use of IFN-α. The Board is asked to take notice of the five case reports where IFN-α associated psychosis is disclosed. There, the IFN-α was given doses that permitted adaption. For example, the following five case reports disclose:

- Bozikas *et al.* IFN-α was administered 3 times a week at 6 million units per dose, and pyschosis appeared at 11 months, see page 136, 2nd col., and page 137, 1st col. (Exhibit E).
- Schaffer *et al.* IFN-α was administered 3 times a week at 5 million units per dose, and pyschosis appeared at 8 months, see page 1102, 2nd col., and page 1103, 1st col. (Exhibit F).
- Taman. IFN- α was administered 3 times a week at 10 million units per dose (abstract), and pyschosis appears at 5 months (Exhibit G).
- Pabaney *et al.* IFN- α was administered daily at 5 million units per dose, and pyschosis appeared at 9 weeks, see page 28, 2nd col. (Exhibit H).
- Ademmer *et al.* IFN-α was administered 3 times a week at 3 million units per dose, and suicidal ideation arose at 4 months, see page 365 (no exhibit-reference was cited by Examiner).

The question of obviousness involves whether the prior art as a whole motivates one of skill to use GRAs to treat IFN-α associated psychosis in patients taking IFN-α. Clearly, when taken as a whole, there is simply no reason to conclude the IFN-α, when given clinically, will increase cortisol levels and induce a psychosis amenable to treatment with GRAs.

The Examiner concludes on page 3 of the Advisory Action by urging that the five case reports are not adequate to "establish that psychotic symptoms that may develop as a result of IFN-α therapy are not related to an increase in cortisol levels." She then repeats that it may still be *possible* that elevated cortisol levels may account for behavioral changes. Each patient may be different. The routes of administration and duration and dosage may affect neuropsychiatric symptoms. Maybe there will be a patient who will develop psychosis before three months. The four case reports do not set a standard.

Once again, each of the Examiner's points avoids addressing the dispositive question at hand. The dispositive question is, does the prior art, when viewed as a combination, suggest to those of skill, the use of GRAs to treat IFN-α associated psychosis? The fact that the case reports fail to establish that psychotic symptoms are not related to cortisol is irrelevant. They

were not intended to establish the fact stated by the Examiner's conclusion. The case reports were presented to demonstrate that the symptoms of psychosis arise well after Gisslinger's reported adaption. Nothing else was implied by the case reports.

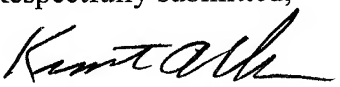
The facts that the various routes of administration, dose, duration of therapy, and patient predisposition might affect symptoms are all true but irrelevant to whether the claimed invention is obvious. The fact that 5 case studies do not constitute a *standard* for the purpose of an FDA clinical trial might be true. But it is all we have before us in the record and the Board's decision with regard *prima facie* case of obviousness must be based on the record — not on possibilities.

Clearly, there are patients who become psychotic after months of IFN-α therapy, and these patients might very well have a sensitivity to elevated levels of cortisol. But the Examiner is wrong when she concludes that these inherent, undisclosed realities set forth a proper *prima facie* case of obviousness. She is using hindsight to reconstruct a story that is not supported by the prior art when the teachings of Gisslinger are taken into account. Indeed, the Examiner's position restates the appellant's inventive contribution. And it is directly contrary to the teachings of the prior art when read as a whole by those of ordinary skill. To conclude that the prior art of record would suggest to one of skill that INF-α associated psychosis was treatable with GRAs in light of Gisslinger's findings of normal cortisol levels in patients treated with IFN-α for three weeks is legally improper. At the very least, the issue before the Board is in equipoise and the claims should be allowed to issue.

8. CONCLUSION

For these reasons, it is respectfully submitted that the rejection should be reversed.

Respectfully submitted,



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9. CLAIMS APPENDIX

The text of the claims involved in the appeal is as follows:

1. (Previously presented) A method of ameliorating the symptoms of psychosis associated with interferon α therapy in a patient, comprising:

administering to the patient having received interferon α therapy and suffering from psychosis associated with the interferon α therapy, an amount of a glucocorticoid receptor antagonist effective to ameliorate the symptoms of psychosis in the patient, with the proviso that the patient is not otherwise in need of treatment with a glucocorticoid receptor antagonist.

2. (Original) The method of claim 1, wherein the glucocorticoid receptor antagonist comprises a steroidal skeleton with at least one phenyl-containing moiety in the 11-beta position of the steroidal skeleton.

3. (Original) The method of claim 2, wherein the phenyl-containing moiety in the 11-beta position of the steroidal skeleton is a dimethylaminophenyl moiety.

4. (Original) The method of claim 3, wherein the glucocorticoid receptor antagonist comprises mifepristone.

8. (Original) The method of claim 1, wherein the glucocorticoid receptor antagonist is administered to the patient concomitantly with interferon-α.

9. (Original) The method of claim 8, wherein the glucocorticoid receptor antagonist is administered to the patient throughout the course of interferon-α therapy.

10. (Original) The method of claim 8, wherein the glucocorticoid receptor antagonist is administered to the patient concomitantly with interferon-α and a second therapeutic agent.

11. (Original) The method of claim 10, wherein the second therapeutic agent is an anti-viral agent.

12. (Previously presented) The method of claim 11, wherein the anti-viral agent is ribavirin.

13. (Original) The method of claim 1, wherein the glucocorticoid receptor antagonist is administered in a daily amount of between about 0.5 to about 25 mg per kilogram of body weight per day.

14. (Original) The method of claim 13, wherein the glucocorticoid receptor antagonist is administered in a daily amount of between about 1 to about 4 mg per kilogram of body weight per day.

15. (Original) The method of claim 1, wherein the mode of administration is selected from the group consisting of oral administration, transdermal application, nebulized suspension, and aerosol spray.

16. (Original) The method of claim 1, wherein the patient is suffering from a viral infection caused by a virus selected from the group consisting of hepatitis C virus, hepatitis B virus, and hepatitis D virus.

17. (Previously presented) The method of claim 16, wherein the viral infection is acute or chronic.

18. (Original) The method of claim 1, wherein the patient is suffering from chronic myelogenous leukemia, HIV, Human T-Cell Lymphotropic Virus or cancer.

19. (Original) The method of claim 1, wherein the patient has a history of substance abuse.

10. EVIDENCE APPENDIX

A. Joseph Belanoff, M.D.– Rule 132 Declaration signed February 10, 2009.

B. Roosth, J. *et al.*, "Cortisol Stimulation by Recombinant Interferon-α_2," *J. Neuroimmunology* **12:** 311-316 (1986).

C. Müller, H. *et al.*, "Interferon-Alpha-2-Induced Stimulation of ACTH and Cortisol Secretion in Man," *Neuroendocrinology* **54:** 499-503 (1991).

D. Gisslinger, H. *et al.*, "Interferon- α Stimulates the Hypothalamic-Pituitary-Adrenal Axis *in vivo* and *in vitro*," *Neuroendocrinology* **57:** 489-495 (1993).

E. Bozikas, V. *et al.*, "An interferon- α-induced psychotic disorder in a patient with chronic hepatitis C," *Eur Psychiatry* **16**: 136-7 (2001).

F. Schäfer, M. *et al.*, "Psychosis in a methadone-substituted patient during interferon-alpha treatment of hepatitis C," *Addiction* **95** (7): 1101-1104 (2000).

G. Pabaney, A. *et al.*, "Interferon α-induced Psychotic Disorder in a Patient with Chronic Hepatitis B," *Medicine Today* **5** (1): 28-30 (Jan.-Mar. 2007).

H. Tamam, L. *et al.*, "Psychosis associated with Interferon alfa therapy for chronic hepatitis B," *Ann Pharmacother* **37** (3): 384-7 (Mar. 2003).

11. RELATED PROCEEDINGS APPENDIX

None

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EXHIBIT A

I hereby certify that this correspondence is EFS-Web with the United States Patent and Trademark office

On 2/13/09

TOWNSEND and TOWNSEND and CREW

By: _____

PATENT

Atty. Docket No.(TTC): 19904-022-1US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 7228
SCHATZBERG & BELANOFF	Examiner: Brooks, Kristie Latrice
Application No.: 10/519,008	Technical Center/Art Unit: 1609
Filed: December 21, 2004	
For: METHODS FOR TREATING PSYCHOSIS ASSOCIATED WITH INTERFERON-ALPHA THERAPY	DECLARATION OF DR. JOSEPH BELANOFF UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Dr. Joseph Belanoff, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true. **Exhibits 1-7,** attached hereto, are incorporated herein by reference.

2. I received an M.D. in 1992 from Columbia University, College of Physicians and Surgeons.

3. I am the inventor of the subject application and I am presently the CEO of Corcept Pharmaceuticals, Inc., the named assignee of the subject application. Corcept's primary mission is to provide improved medicine for psychiatric illnesses.

4. I have read and am familiar with the contents of the application. I understand that the Examiner has rejected the pending claims under §103 based upon her belief that one of skill reading the prior art of Schatzberg (U.S. Pat. No. 6,150,349) in view of Ademmer and/or Dieterich and Shimizu would have a reasonable expectation that patients with psychosis induced by IFN-α would be treatable with glucocorticoid receptor antagonist [GRA].

5. In the previous Office Action mailed on May 7, 2008, the Examiner based her rejections upon the combination of Schatzberg and Ademmer or Dieterich. According to the Examiner, Schatzberg taught that glucocorticoid receptor antagonists [GRAs] can be used to treat psychosis due to glucocorticoid dysregulation, and Ademmer and Dieterich disclose psychotic episodes in persons taking IFN-α for chronic conditions. It was explained in our response mailed on August 22, 2008 that the combination of references failed link IFN-α induced psychosis with glucocorticoid dysregulation.

In the final office action the Examiner cited to Shimizu as teaching that IFN-α can elevate cortisol. The obviousness rejection was maintained.

6. Shimizu by itself does not tell the full story of the impact of IFN-α on the hypothalamic pituitary adrenal axis [HPA]. Shimizu is following up on the earlier work of Roosth *et al.* in 1986 (**Exhibit 1**) where they report that following injections of IFN-α cortisol levels increase but return to normal within 24 hours. On page 315, the authors note that the phenomenon was transient. There was thought that the elevation in cortisol might be due to the transient fever that often accompanies the administration of IFN-α (see table 3).

In 1993, Muller *et al.* (**Exhibit 2**) looked at the mechanism of action for the reports of IFN-α elevation of cortisol and ACTH. They emphasize the possibility that

fever might be the cause (page 499, 1st column). They note in the first sentence of the abstract on page 499, that the observed effect is "short term" and these studies were confined to measuring cortisol in the first 24 hours after administration. The results of Muller confirm the results of Roosth and Shimizu. The IFN-α induced cortisol elevation is transient with maximal levels arising after 5.8 hours (see table 2 on page 502). They also concluded that temperature elevations were not likely the cause of the transient elevation.

IFN-α therapy is often used as a long term therapy for chronic conditions. In 1993, Gisslinger *et al.* (**Exhibit 3**) looked at patients treated with IFN-α for three weeks and concluded that the transient elevation of cortisol was actually temporary and after 3 weeks, the cortisol elevation disappeared. Gisslinger called it "IFN-α –induced adaptive changes in the HPA" (see abstract). They wrote in their abstract, " After three weeks of IFN-α therapy, no significant stimulation of the HPA was observed."

7. Having demonstrated that the Shimizu disclosure of IFN-α stimulated HPA is reporting on a temporary phenomenon and that disappears after 3 weeks, the remaining question is whether the IFN-α –induced psychosis is present within this 3 week window. It is not. IFN-α-induced psychosis is observed in <1.0% of the patients taking the drug and arises only after months of therapy. Thus, our method of treatment claims address a disease that is clearly outside the 3 week window where IFN-α elevates cortisol.

Evidence that IFN-α –induced psychosis arises after months of IFN-α therapy can be found in the literature. See **Exhibits 4-7:** Bozikas 2001 (**11 months** of continuous treatment); Shafer 2000 (**4 months**), Taman 2003 (**5 months**) and Pabaney 2007 (**4 months**).

8. It is my opinion that one of skill reading Schatzberg, Ademman, Dieterich and Shimizu **in view of Gisslinger and the reports of exhibits 4-7** would not be motivated to consider GRAs as an effective therapy for treating IFN-α –induced pyschosis. The combination of references clearly suggests away from my invention.

This Declarant has nothing further to say.

Dated: 2/10/09

Joseph Belanoff, M.D.

Attachment: Exhibits 1-7

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EXHIBIT B

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Cortisol Stimulation by Recombinant Interferon-α_2

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(Accepted 2 April, 1986)

Summary

Serum cortisol concentrations were determined by radioimmunoassay in cancer patients undergoing experimental therapy with recombinant interferon-α_2. Cortisol concentration rose steadily after interferon administration and was significantly different from that on control day at 8 h following intramuscular injection of interferon-α_2. Cortisol elevation was increased as weekly doses of interferon were increased ($0–120 \times 10^6$ units). Recent clinical trials of interferons for treatment of neurological and malignant diseases provide a compelling need to understand the actions and side effects of exogenously administered interferons.

Key words: *Cortisol stimulation – Interferon-α_2, recombinant – Serum – Tumor*

Introduction

Interferons are a class of proteins which act by inducing resistance to viral infection and by modulating the immune response (Friedman and Vogel 1970). It has recently been proposed that interferons may induce their effects via pathways similar to those of hormones (Blalock and Stanton 1980; Smith and Blalock 1981); and it has been demonstrated that like adrenocorticotropic hormone (ACTH),

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interferon stimulates a dose-dependent steroidogenic response by mouse adrenal tumor cells (Blalock and Harp 1981). The existence of a lymphoid-adrenal axis is suggested by the enhanced production of corticosteroids in normal (Solomon et al. 1967) and hypophysectomized mice (Smith et al. 1982) treated with interferon-inducing agents such as viruses. Likewise, humans who have undergone pituitary stalk resection (Van Wyk et al. 1960) respond to bacterial polysaccharide with elevation of the cortisol concentration.

The recent clinical trials of interferons for treatment of multiple sclerosis (Jacobs et al. 1981; Knobler et al. 1984) and of metastatic disease (Priestman 1983; Kirkwood and Ernstoff 1984) underscore the need for clarifying the in vivo consequences of administration of interferons to patients. Measurement of the plasma cortisol concentrations in individuals undergoing clinical trials with interferon-α_2 therapy for solid tumors demonstrated an increase in cortisol concentrations in these patients over a 24 h period.

Materials and Methods

Patients

The seven patients in this study ranged in age from 34 to 67 years with a mean ($\pm$SD) of 54.7 ± 12.6 years. All patients were informed and consented to experimental therapy for solid tumors with recombinant interferon-α_2 (Schering Corp., Bloomfield, NJ). Intramuscular injections of 0 (the diluent alone containing normal saline with 1 mg/ml of human serum albumin), 1, 10, 30, 60, or 120×10^6 units of recombinant interferon-α_2 were administered in ascending order to patients between 8:00 and 9:00 a.m. at weekly intervals over a 7-week period. Blood was drawn immediately prior to (0 h) and 1, 2, 4, 8 and 24 h post-injection. Two additional patients were studied hourly for a 24 h period after receiving injections of 10×10^6 units of interferon-α. These studies confirmed that sampling in other patients was adequate. Serum was frozen at $-70°C$ until cortisol assays were performed.

Because of the pyrogenic effects of interferon, patients received 650 mg of acetominophen (Tylenol) prior to and every 4 h post-treatment. The oral temperature of these patients was closely monitored.

Cortisol assays

Cortisol levels were determined by radioimmunoassay of ethanol-extracted serum samples. Antibody to cortisol was produced in rabbits (Cambridge Nuclear, Cambridge, MA) and [^{3}H]1,2,6,7-hydrocortisone (New England Nuclear, Boston, MA) was used as ligand. The complex was precipitated utilizing polyethylene glycol (Sigma Chemical Co., St. Louis, MO) in a modification of the technique described by Desbuquois and Aurbach (1971). Parallel dose-response curves for experimental serum samples and a cortisol standard were demonstrated over a 10-fold range. The intra-assay variability was 5.4% and the interassay variability was 5%.

Results

Timed measurements of cortisol concentrations in patients receiving interferon-α_2

The plasma cortisol concentrations in five patients injected intramuscularly with 120×10^6 units of recombinant interferon-α_2 were determined over a 24 h period. Fig. 1 illustrates the results of these experiments and compares them to four of the group when they received placebo. While the cortisol concentrations in patients receiving interferon-α_2 did not differ from placebo levels at 0 h, a trend toward elevated plasma cortisol concentrations in interferon-α_2-treated patients was evident at 2 and 4 h. By 8 h, cortisol concentrations in the placebo group ranged from 6.5 μg/dl to 13.3 μg/dl ($n = 4$) whereas in the group receiving interferon, cortisol concentrations ranged from 21.0 μg/dl to 48.1 μg/dl ($n = 5$); the measure was significantly different (8.6 ± 1.6 μg/dl and 31.3 ± 5.4 μg/dl respectively, $P < 0.005$). By 24 h post-injection, the cortisol concentrations in all patients had returned to pretreatment levels (12.2 ± 3.4 μg/dl). Two patients studied hourly documented that the peak cortisol concentrations occurred at 7 and 8 h after interferon injection (data not shown).

The oral temperature of these patients was monitored after placebo or interferon administration to assess the relationship of body temperature and cortisol concentration (Table 1). Prior to placebo injections, the mean ($\pm$SD) temperature was 36.9 ± 0.7°C and at 8 h had risen to 37.2 ± 0.2°C ($P > 0.05$, $n = 4$). On the day of interferon treatment, patients had temperatures of 36.6 ± 0.8°C prior to interferon injection and of 37.8 ± 0.8°C at 8 h ($P < 0.02$, $n = 5$). Although there was a

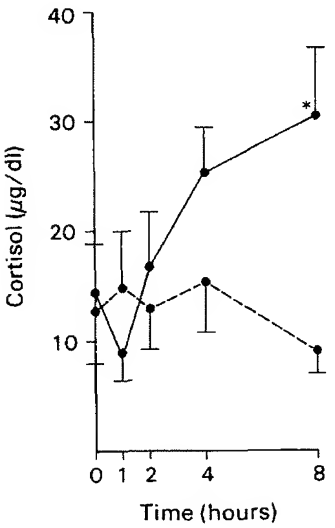

Fig. 1. Interferon-α_2 increases cortisol concentration in patient serum. Mean cortisol concentration in serum from patients receiving placebo (●- - - - -●, $n = 4$) or 120×10^6 units of interferon-α_2 (●———●, $n = 5$) are shown at various times post-inoculation. The Student t-test was used to determine significance (* $P < 0.005$) between the interferon and placebo groups for each time post 0 h.

TABLE 1

ORAL TEMPERATURE OF PATIENTS RECEIVING PLACEBO OR 120×10^6 U INTERFERON-α_2 [a]

	Time (h)				
	0	1	2	4	8
Placebo	36.9±0.7 [b]	37.0±0.3	36.9±0.4	37.2±0.2	37.2±0.2
120×10^6 U IFN-α_2	36.6±0.8	36.7±0.5	36.6±0.5	37.2±0.5	37.9±0.8

[a] These data are from the patients illustrated in Fig. 1. For placebo, $n = 4$ and for interferon-α_2 (IFN-α_2) treatment, $n = 5$.

[b] Data are expressed as mean ± SD.

significant difference in temperatures of patients at 8 h when compared to their 0 h temperatures, there was no significant difference between patients 8 h after receiving placebo and 8 h after receiving 120×10^6 units of interferon-α_2 ($P > 0.05$).

Cortisol concentrations are elevated by increasing doses of interferon

Because the peak effects on cortisol concentration regardless of dose of interferon-α_2 were seen at 8 h post-inoculation, this time point was chosen to delineate the effect of increasing doses of interferon-α_2 on glucocorticosteroid concentration. Table 2 shows the effects of varying doses of interferon-α_2 in seven patients on serum cortisol concentration 8 h post various doses of interferon. An elevation of cortisol was observed in all patients tested. For example, subject No. 5 exhibited a cortisol concentration of 8.8 μg/dl 8 h after placebo, 20.1 μg/dl after 10×10^6 units of interferon-α_2, and 48.8 μg/dl after 120×10^6 units of interferon-α_2. The dose of interferon which elicited the peak cortisol response differed in individual patients, but cortisol was elevated in all patients. When compared to the placebo group, the serum cortisol concentrations differed significantly in patients treated with 10×10^6 to 120×10^6 units of interferon-α_2 ($P < 0.05$ by one-way analysis of variance).

TABLE 2

SERUM CORTISOL (μg/dl) 8 h AFTER VARYING DOSES OF INTERFERON-α_2

Patient No.	Units of interferon-α_2 ($\times10^6$)					
	0 (placebo)	1	10	30	60	120
1	6.2	12.7	31.0	n.d. [a]	23.2	23.3
2	15.2	15.2	n.d.	n.d.	32.7	39.8
3	11.8	13.8	18.9	35.2	34.5	n.d.
4	9.2	17.0	30.8	39.1	31.7	32.2
5	8.8	n.d.	20.1	n.d.	n.d.	48.1
6	7.6	32.7	30.6	19.2	19.3	21.0
7	7.6	9.4	31.4	n.d.	n.d.	n.d.
Mean ± SE	9.5±1.2	16.8±3.4 [b]	27.1±2.4 [b]	31.2±6.1 [b]	28.3±3.0 [b]	32.9±5.1 [b]

[a] n.d. = not done.

[b] $P < 0.05$ by one-way analysis of variance when compared to placebo.

Discussion

Patients treated with 120×10^6 units recombinant interferon-α_2 exhibited significantly elevated concentrations of serum cortisol within 8 h of treatment compared to placebo-treated patients. An enhanced serum cortisol concentration was no longer observed by 24 h post-treatment, indicating that the effect was transient. When patients were treated with increasing doses of interferon-α_2, there was a correspondingly enhanced concentration of cortisol.

It is not clear whether interferon-α_2 acts directly on adrenal cells to stimulate steroidogenesis, as does ACTH. The time course of stimulation is long (lasting many hours) compared to the rather rapid 2 h response seen in patients given intravenous synthetic $ACTH_{1-24}$ for assessment of adrenal function (Chamberlin and Meyer 1981). The acute rise after ACTH to a peak of 25 μg/dl to 35 μg/dl usually occurs between 30 min to 2 h after injection (Lee et al. 1973). It is also possible that interferon-α_2 mediates steroidogenic effects indirectly by stimulating pituitary or extra-pituitary release of ACTH. Another possibility which cannot yet be excluded is that the stress of fever may have contributed to the enhanced serum cortisol levels observed in these patients. However, the former explanation appears unlikely, because the oral temperature of patients receiving interferon did not differ significantly from those receiving placebo. That nonspecific stressors such as myalgias, arthralgias or fatigue caused cortisol elevation also seems unlikely since there appeared to be no difference in cortisol elevation between those patients reporting moderate to severe side effects when compared to those exhibiting mild side effects or having none. Regardless of the mechanism of interferon-α-action, cortisol concentrations in serum are significantly enhanced by 8 h post-injection.

Recent studies have indicated that interferon may have a steroidogenic effect when used at concentrations exceeding those required for an antiviral effect (Blalock and Harp 1981). The in vivo administration of agents which elicit an interferon response has also been reported to stimulate steroidogenesis, as indicated by elevated corticosteroid concentrations in both intact and hypophysectomised animals (Van Wyk et al. 1960; Solomon et al. 1967; Smith et al. 1982).

The direct or indirect steroidogenic effects of recombinant interferon-α_2 need to be documented carefully, because interferon therapy is now being tested in a variety of human diseases. Numerous side effects of interferon therapy, including neuropsychiatric manifestations (Adams et al. 1984), have been reported. Theoretically it is possible that elevated cortisol concentrations may account for behavioral changes. In addition, because glucocorticoids are powerful immune modulators (Claman 1975; Cupps and Fauci 1982) and the circadian variation in corticoid levels correlates closely with a bioperiodicity of the immune response (Abo et al. 1981; Kawate et al. 1981), careful timing of interferon treatment may allow enhancement of its therapeutic effects.

Acknowledgements

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References

Abo, T., T. Kawate, K. Itoh and K. Kumagai, Studies on the bioperiodicity of the immune response: circadian variations of human T, B, and K traffic in the peripheral blood, J. Immunol., 126 (1981) 1360–1363.

Adams, F., J.R. Quesada and J.V. Gutterman, Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer, J. Am. Med. Assoc., 252 (1984) 938–941.

Blalock, J.E. and C. Harp, Interferon and adrenocorticotropic hormone induction of steroidogenesis, melanogenesis, and antiviral activity, Arch. Virol., 67 (1981) 45–49.

Blalock, J.E. and J.D. Stanton, Common pathways of interferon and hormonal action, Nature, 283 (1980) 406–408.

Chamberlin, P. and W.J. Meyer, The management of pituitary-adrenal suppression secondary to corticosteroid therapy, Pediatrics, 67 (1981) 245–251.

Clamen, H.N., How corticosteroids work, J. Allergy Clin. Immunol., 55 (1975) 145–151.

Cupps, T.R. and A.S. Fauci, Corticosteroid mediated immunoregulation in man, Immunol. Rev., 65 (1982) 133–155.

Desbuquois, B. and G.D. Aurbach, Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassays, J. Clin. Endocrinol. Metab., 33 (1971) 732–738.

Friedman, R.M. and S.N. Vogel, Interferon with special emphasis on the immune system, Adv. Immunol., 34 (1983) 97–138.

Jacobs, L., J. O'Malley, A. Freeman and R. Ekes, Intrathecal interferon reduces exacerbations of multiple sclerosis, Science, 214 (1981) 1026–1028.

Kawate, T., T. Abo, S. Hinuma and K. Kumagai, Studies on the bioperiodicity of the immune response. Covariations of murine T and B cells and a role of corticosteroid, J. Immunol., 126 (1981) 1364–1367.

Kirkwood, J.M. and M.S. Ernstoff, Interferons in the treatment of human cancer, J. Clin. Oncol., 2 (1984) 336–352.

Knobler, R.L., H.S. Panitch, S.L. Braheny, J.C. Sipe, G.P.A. Rice, J.R. Huddlestone, G.S. Francis, C.K. Hooper, R.M. Kamin-Lewis, K.P. Johnson, M.B.A. Oldstone and T.C. Merigan, Systemic alpha interferon therapy of multiple sclerosis, Neurology, 34 (1984) 1273–1279.

Lee, P.A., B.S. Keenan, C.J. Migeon and R.M. Blizzard, Effect of various ACTH preparations and of metyrapone on the secretion of growth hormone in normal subjects and in hypopituitary patients, J. Clin. Endocrinol. Metab., 37 (1973) 389–396.

Priestman, T.J., Interferons and cancer therapy, J. Pathol., 141 (1983) 287–295.

Solomon, G.F., T.C. Merigan and L. Levine, Variation in adrenal cortical hormones within physiologic ranges, stress, and interferon production in mice, Proc. Soc. Exp. Biol. Med., 126 (1967) 74–79.

Smith, E.M. and J.E. Blalock, The hormonal nature of the interferon system, Tex. Rep. Biol. Med., 41 (1981) 350–358.

Smith, E.M., W.J. Meyer and J.E. Blalock, Virus induced corticosterone in hypophysectomized mice: a possible lymphoid adrenal axis, Science, 218 (1982) 1311–1312.

Van Wyk, J.J., G.S. Dugger, J.F. Newsome and P.Z. Thomas, The effect of pituitary stalk section on the adrenal function of women with cancer of the breast, J. Clin. Endocrinol. Metab., 20 (1960) 157–172.

EXHIBIT C

Neuroendocrinology 1991;54:499–503

0028–3835/91/0545–0499 $ 2.75/0

Interferon-Alpha-2-Induced Stimulation of ACTH and Cortisol Secretion in Man

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[a]Department of Psychiatry and [b]1st Clinic for Internal Medicine, Johannes Gutenberg University of Mainz, FRG

Key Words. Interferon-α_2 · ACTH · Cortisol · Hepatitis B · Neuroimmune interactions

Abstract. Short-term effects of interferon-α_2 on plasma concentrations of adrenocorticotrophic hormone (ACTH) and cortisol were measured in man in relation to interferon absorption. Interferon-α_2 was given subcutaneously at a dose of 3×10^6 IU at 17.00 h to 2 female and 5 male patients who suffered from chronic hepatitis B infection and who had not previously been treated with interferon. Plasma levels of ACTH, cortisol and interferon-α were determined at 30-min intervals between 16.00 and 24.00 h. In each patient a similar cortisol, ACTH and interferon-α profile was determined on a day, when no interferon-α treatment was given. Interferon-α plasma levels peaked around 21.30 h, i.e. 4.7 h after injection. In each patient ACTH and cortisol levels were increased. As calculated from the areas under the curves, ACTH release was increased by an average of 332% (maxima at about 22.00 h, i.e. 5.2 h post injection); cortisol release was increased by an average of 311% (maxima at about 23.00 h, 5.8 h post injection). These actions were not related to side effects like fever or other flu-like symptoms. Our findings confirm that in man as in animals interferon-α_2 can act as a mediator between the immune and endocrine system.

There is evidence of bidirectional communication between the immune system and the hypothalamo-pituitary-adrenal (HPA) axis. Glucocorticoids inhibit or enhance immune functions, depending on concentration, time of application, cell type and species [1]. On the other hand, immunomodulatory substances can stimulate the HPA axis [2, 3]. The latter has been shown primarily in animals. Few investigations have studied the hormonal effects of cytokines in man with inconsistent results. Interleukin-2, interferon-γ or -α_2 have been shown to stimulate the secretion of cortisol in man [3–7]. Effects of the secretion of adrenocorticotrophic hormone (ACTH) have been registered in two studies using interferon-γ [5, 6] which did not significantly interfere with the release of ACTH. Moreover, from the data reported so far it cannot be excluded that stimulation of the HPA axis by cytokines is due to fever or other flu-like symptoms which may occur after administration of cytokines [8].

Interferon-α_2 is not only a physiologically relevant pleiotrophic lymphokine that is produced by monocytes or other leukocytes [9] but it is also a new therapeutic agent that is currently under investigation for the treatment of a number of diseases. Therapeutic efficiency has been shown for cancer [10], leukemia [11], hepatitis [12] or AIDS-associated Kaposi sarcoma [13].

The present investigation included patients suffering from chronic hepatitis B infection that had been selected for treatment with interferon-α_2. It was the aim of the study to look for time-dependent alterations in the secretion of ACTH and cortisol after drug administration in these patients. Moreover, skin temperature and heart rate were carefully monitored and the plasma levels of interferon-α were determined in identical blood samples withdrawn for the determination of ACTH and cortisol. Simultaneous quantification of hormone and interferon-α concentrations in the circulation have not been reported so far.

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Patients and Methods

Patients

The study included 7 patients suffering from chronically active hepatitis B infection who were in a clinically stable state without signs of decompensation. They were treated with 3×10^6 IU recombinant human interferon-α_2 (Roferon A 3, Hoffmann-La Roche, Basel, Switzerland) 3 times a week for 4 months. Two women and 5 men, aged 26–69 years (mean ± SD; 49 ± 16.2; median, 53), were included. Written informed consent was obtained from all patients. The study protocol had been approved by the hospital ethics council.

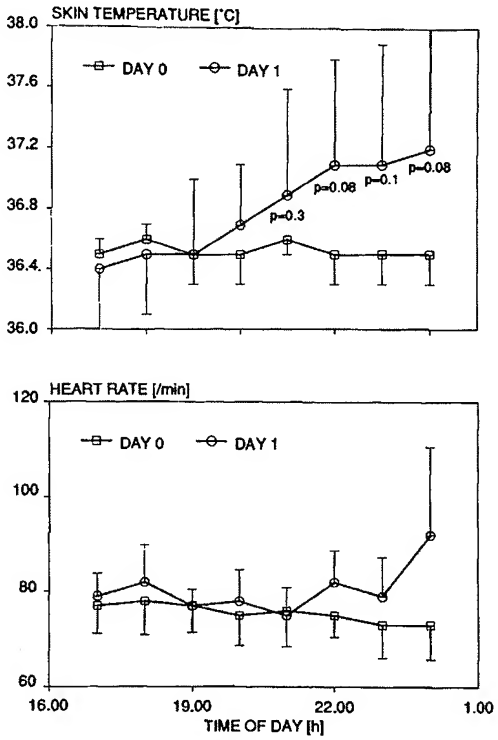

Fig. 1. Skin temperatures and heart rates in patients who received subcutaneous doses of 3×10^6 IU interferon-α_2 on day 1. Baseline measurements were performed on day 0, the day before drug administration. Values given are the mean ± SD obtained from 7 patients. Indicated p values were calculated by statistical comparison (ANOVA) of temperatures of day 0 and day 1.

Collection of Blood

On the day before treatment (day 0), and on the first day of treatment (day 1), blood was withdrawn between 16.00 and 24.00 h every 30 min for determination of ACTH, cortisol and interferon-α. Because of the chronic liver diseases and possible differences in the metabolism of hormones each patient was used as his own control on day 0 for registration of baseline secretion of hormones and interferon-α. At 17.00 h on day 1, a subcutaneous dose of 3×10^6 IU interferon-α_2 was injected. The time schedule of investigation was selected to reduce interferences with the well-known diurnal variations in cortisol and ACTH production.

To reduce stress effects, patients rested in bed from 15.00 up to 24.00 h. At 15.30 h a catheter was inserted into a forearm vein and kept open with physiological saline (30 ml/h). Blood was drawn through the catheter into chilled tubes which contained heparin for cortisol and interferon-α determinations or ethylenediaminetetracetic acid (EDTA, 1 mg/ml) and 400 kIU trasylol for ACTH determinations. Plasma was prepared by centrifugation of the blood at 4 °C and it was stored frozen (–20 °C) until assayed.

Physiological Measurements

Blood pressure, heart rate and body temperature in the axilla were recorded every 60 min. Patients were requested to report flu-like symptoms every hour.

Determination of Hormones

ACTH and cortisol were determined using commercial radioimmunoassay kits: cortisol from Becton Dickinson, Heidelberg, FRG and ACTH from Nichols Institute, Bad Nauheim, FRG. The ACTH assay has a detection limit of 2 pg/ml and does not cross-react with ACTH fragments such as $ACTH_{1-3}$ (melanotropin), $ACTH_{1-17}$, $ACTH_{1-14}$, $ACTH_{34-39}$, endorphin or lipotropin [14]. The intra-assay coefficients of variation were 3–4% for cortisol and 5% for ACTH. Each series was analyzed in a single run to avoid interassay variations.

Interferon Assay

Interferon-α concentrations were determined with an enzyme-linked immunoassay as described previously [15]. This assay is based on the procedure established by Gallati [16]. It uses a monoclonal antibody that recognizes both interferon-α subtypes: interferon-α_1 and -α_2. The concentrations of interferon-α were calculated by comparing the extinctions of the samples with those obtained in assays with known interferon-α concentrations corresponding to the standard No. Gxa01-901-535 of the National Institutes of Health (NIH).

Statistical Test

Areas under curves (AUC) for ACTH and cortisol secretion over time were calculated from 18.30 to 24.00 h. Significance of differences in mean heart rates or skin temperatures after drug administration was determined by analysis of variance (ANOVA) and in hormone secretion between treated (day 0) and nontreated (day 1) patients by a paired t test. Differences were considered as significant for $p < 0.05$.

Results

Clinical Symptoms

The subcutaneous injections of interferon-α_2 were generally well tolerated and the low dose caused few side effects (fig. 1). Only 3 of the 7 patients developed temperatures exceeding 37 °C. In 1 patient it increased to 38.6 °C. The heart rates rose in those 3 patients, in 2 of them up to 120/min. Moreover, the 3 patients also complained of mild flu-like symptoms such as arthralgia, myalgia and fatigue. Blood pressure remained constant. There were no significant differences in temperature, heart rate or systolic blood pressure between day 0 and day 1 (fig. 1).

Interferon-α

Plasma levels of interferon-α began to increase within 1.5–3.5 h (mean ± SD, 1.9 ± 0.7; median, 1.5) with peaks at 2–7 h (mean ± SD, 4.7 ± 1.4; median, 5) after subcutaneous injection of interferon-α_2 at 17.00 h (table 1). Plasma peaks for interferon-α ranged from 1.0 to 11 IU/ml and plasma levels remained elevated until the end of the investigation at 24.00 h. In 3 noninjected patients the levels of interferon-α were up to 1.5 IU/ml (fig. 2).

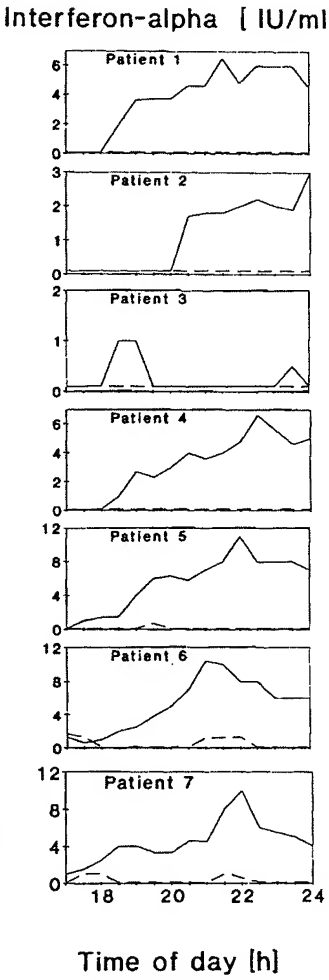

Fig. 2. Plasma levels of interferon-α in 7 patients injected with 3 × 10^6 IU interferon-α_2 at 17.00 h (——). Baseline levels were measured on the day before drug administration (— — —). Blood was withdrawn at 30-min intervals for the preparation of plasma samples.

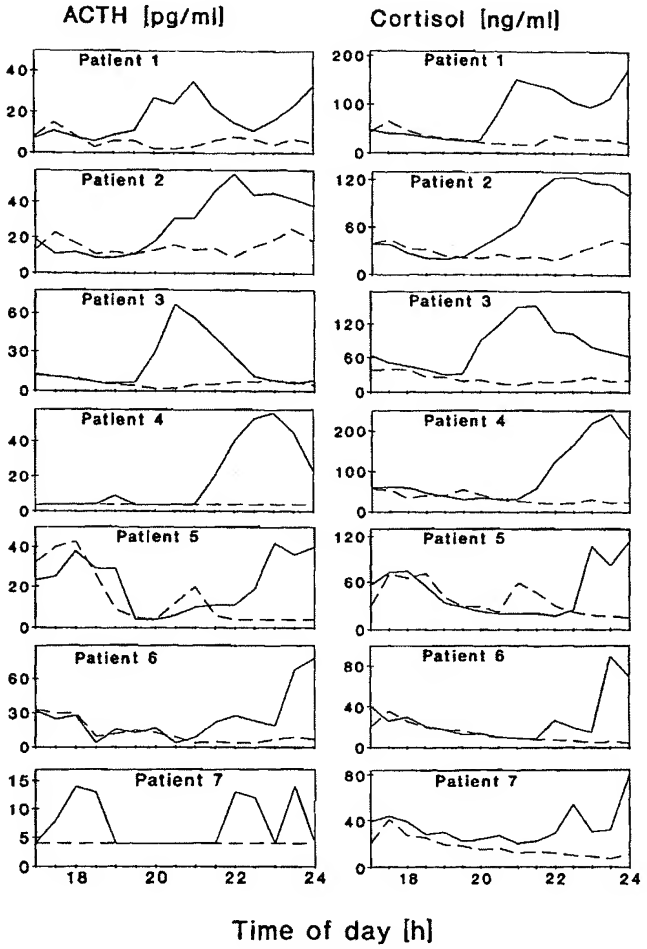

Fig. 3. Concentrations of ACTH and cortisol in the plasma of 7 patients who received subcutaneous injections of 3 × 10^6 interferon-α_2 (——) at 17.00 h. Baseline levels (— — —) were measured on the day before drug administration. For determination of hormonal profiles blood samples were withdrawn at 30-min intervals.

ACTH and Cortisol

The elevated plasma levels of interferon-α correlated with enhanced ACTH and cortisol secretion in each patient. It seemed unlikely that the hormonal responses to interferon-α were the result of nonspecific side effects such as the increase in heart rate or body temperature (fig. 1). The onset of the side effects was measurable later than the increase in hormone release. Moreover, the patients exhibiting the highest cortisol (patient 4) or ACTH (patient 6) response did not show those side effects. The beginning of the rise in ACTH release was highly variable; it occurred between 0 and 4 h after the increase in interferon-α plasma levels (mean ± SD, 2.1 ± 1.2; median, 2.5). Plasma peaks occurred between 3.5 and 7 h after interferon-α_2 injection (mean ± SD, 5.2 ± 1.1; median, 5) (table 1) ranging between 14 and 78 pg/ml (fig. 3). Cortisol followed ACTH increase within 0–30 min with plasma peaks between 4 and 7 h (means ± SD, 5.8 ± 1.1; median, 6.5) after interferon-α_2 injection (table 1). Peaks ranged from 81 to 242 ng/ml (fig. 4). There was no correlation of the height of interferon-α and ACTH or cortisol peaks. Some patients exhibited a slight increase in ACTH and cortisol immediately after inserting the needle (fig. 3). The plasma levels of ACTH and cortisol after interferon-α_2 application were significantly elevated between 18.30 and 24.00 h ($p < 0.05$, paird t test), as calculated from areas under curves (fig. 4).

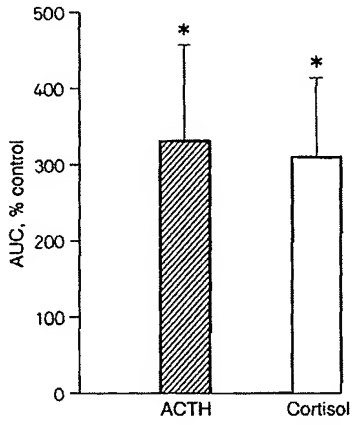

Fig. 4. Integrated AUC of ACTH and cortisol in the plasma of patients who were injected with interferon-α_2 as described in the legend to figure 3. AUC values were calculated from hormone levels between 18.30 and 24.00 h and related to the respective AUCs obtained from the hormone concentrations of the day before drug administration. Values given are the mean ± SD obtained from 7 patients. *$p < 0.05$, vs. control (paired t test).

Table 2. Maximal ACTH and cortisol response after interferon-α_2 administration in relation to maximal interferon-α levels in the plasma

Patient No.	Time of maximal plasma levels after s.c. injection of 3 × 10^6 IU IFN-α_2 for		
	IFN-α	ACTH	Cortisol
1	4.5	4.0	4.0
2	7.0	5.0	5.5
3	2.0	3.5	4.5
4	5.5	6.0	6.5
5	5.0	6.0	7.0
6	4.0	7.0	6.5
7	5.0	5.0	7.0
Mean value ± SD	4.7 ± 1.4	5.2 ± 1.1	5.8 ± 1.1

Values given are hours when plasma peaks occurred in individual patients and means ± SD. IFN = Interferon.

Discussion

The results show that acute doses of interferon-α_2 stimulate ACTH and cortisol secretion in man. The time pattern of ACTH increase observed after injection of interferon-α_2 was highly variable. Roosth et al. [7] and Scott et al. [17] reported an increase in cortisol production after intramuscular injections of interferon-α_2 in the morning with peak values after 8 h. This differs from our results in which cortisol peaks occurred between 4 and 7 h after injection. The difference might be due to time-dependent variations in the sensitivities of the ACTH/cortisol release system towards interferon-α_2. We chose the afternoon for interferon-α_2 application, since normally the release of HPA hormones remains rather constant between 16.00 and 2.00 h. ACTH peaks are rare between 18.00 and 24.00 h [18].

It seemed unlikely that the effects on ACTH and cortisol release were due to nonspecific stress, enhanced body temperature, heart rate or blood pressure, because only 3 of the 7 patients exhibited an increase in body temperature or heart rate whereas all patients showed elevated ACTH and cortisol plasma levels. This is in agreement with findings of other investigators who also failed to see significant changes in body temperature after interferon treatment [7] whereas Scott et al. [17] found an increase in temperature in all patients. Moreover, stress effects brought about by insertion of the needle are much more rapid in onset than the observed increases in ACTH and cortisol that were measurable after injection of interferon-α_2 and after the rise in interferon-α plasma levels.

There is little information about the mechanism of interferon-α stimulation of the HPA axis. Since interferon-α_2 is a polypeptide unable to penetrate the blood brain barrier, it is unlikely that it can enter the brain [19]. Smith et al. [20] could not find measurable concentrations of recombinant interferon-αA in the cerebrospinal fluid (CSF) of subjects within 48 h after intravenous injection of 18 × 10^6 IU recombinant interferon-αA. Only an infusion of 50 × 10^6 IU lead to a small increase in recombinant interferon-αA within the CSF of 3 of 4 patients. Rohatiner et al. [21] found low interferon-α levels in the CSF in only 1 of 5 patients who received 100 × 10^6 IU/m^2/day.

A second interpretation that must be considered concerns ACTH-like activity of interferon-α due to a structural relationship to ACTH as proposed by Blalock and Smith [22] thus interfering with the in vitro determination of ACTH. Blalock and Smith [23] used antibodies against the N-terminal end of $ACTH_{1-13}$. We used a two-site immunoradiometric assay. The assay detects only intact ACTH but not ACTH fragments such as $ACTH_{1-13}$ (melanotropin), $ACTH_{1-17}$, $ACTH_{1-24}$, $ACTH_{34-39}$, endorphin of lipotropin [14]. Moreover, in our study there was no quantitative correlation between interferon-α and ACTH. For example in patient 3 there was only a small increase in interferon-α (fig. 2) but a large increase in ACTH (fig. 3). On the other hand, patient 7 (fig. 2, 3) had high interferon levels but only a mild increase in ACTH.

The third possible mechanism that might be involved is the production of ACTH by lymphocytes. Lymphocytes are able to express the proopiomelanocortin gene and secrete ACTH [24, 25]. However, the time interval between stimulation of genomic activity of leukocytes by corticotropin-releasing hormone and maximal de novo synthesis of ACTH is 48 h [24], while the effects reported here were much more rapid.

Moreover, it must be assumed that some lymphokines seem to have CRH-like activity [26] although McGillis et al. [27] did not find ACTH release from cultured rat pituitary cells after stimulation with different cytokines including interferon-α. From the literature reported so far, it can therefore not yet be decided at which level lymphokines act upon the HPA axis. In spite of this limitation, however, it seems likely that alterations in HPA activity after cytokine treatment play a physiological role because different cytokines have distinct effects on the HPA axis: after application of low or high doses of interferon-γ, we and others found a cortisol increase without ACTH increase [5, 6] whereas acute doses of interferon-α_2 stimulated both, ACTH and cortisol release, a result similar to those seen in lower animals. The observed time pattern of hormone response seems more likely to be the outcome of a releasing effect on ACTH, or a preceding mediator, than of alterations in de novo synthesis.

References

1 Hall NR, Goldstein AL: Endocrine regulation of host immunity: The role of steroids and thymosin; in Feniche RL, Chirigos AM (eds): Immune Modulation Agents and Their Mechanisms. New York, Marcel Dekker 1984, pp 533–563.

2 Buzzetti R, McLoughlin L, Scavo D, Rees LH: A critical assessment of the interactions between the immune system and the hypothalamo-pituitary-adrenal axis. J Endocrinol 1989;120:183–187.

3 Smith EM: Hormonal activities of lymphokines, monokines, and other cytokines; in Blalock JE, Bost KL (eds): Neuroimmunoendocrinology. Prog Allergy. Basel, Karger 1988, vol 43, pp 121–139.

4 Lotze MT, Frana LW, Sharrow SO, Robb RJ, Rosenberg SA: In vivo administration of purified human interleukin 2. I. Half-life and immunologic effects of the jurkat cell line-derived interleukin 2. J Immunol 1985;134:157–166.

5 Späth-Schwalbe E, Porzsolt F, Digel W, Born J, Kloss B, Fehm HL: Elevated plasma cortisol levels during interferon-γ treatment. Immunopharmacology 1989;17:141–145.

6 Holsboer F, Stalla GK, von Bardeleben U, Hammann K, Müller H, Müller OA: Acute adrenocortical stimulation by recombinant gamma interferon in human controls. Life Sci 1988;42:1–5.

7 Roosth J, Pollard RB, Brown SL, Meyer WJ III: Cortisol stimulation by recombinant interferon-α_2. J Neuroimmunol 1986;12: 311–316.

8 Fent K, Zbinden G: Toxicity of interferon and interleukin. Trends Pharmacol Sci 1987;8:100–104.

9 Sen GC: Biochemical pathways in interferon-action. Pharmacol Ther 1984;24:235–257.

10 Okita K, Kaneko T: The potential of interferons in malignant disease. Drugs 1990;39:1–6.

11 Berman E, Heller G, Kempin S, Gee T, Tran LL, Clarkson B: Incidence of response and long-term follow-up in patients with hairy cell leukemia treated with recombinant interferon alfa-2a. Blood 1990;75:839–845.

12 Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH: Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321:1506–1510.

13 Groopman JE, Scadden DT: Interferon therapy for Kaposi sarcoma associated with the acquired immunodeficiency syndrome (AIDS). Ann Intern Med 1989;110:335–337.

14 Zahradnik R, Brennan G, Hutchison JS, Odell WD: Immunoradiometric assay of corticotropin with use of avidin-biotin separation. Clin Chem 1989;35:804–807.

15 Rossol S, Voth R, Laubenstein HP, Müller WEG, Schröder HC, Meyer zum Büschenfelde KH, Hess G: Interferon production in patients infected with HIV-1. J Infect Dis 1989;159:815–821.

16 Galatti H: Interferon. A greatly simplified enzyme-immunological determination using two monoclonal antibodies. J Clin Chem Biochem 1982;20:907–914.

17 Scott GM, Ward RJ, Wright DJ, Robinson JA, Onwubalili JK, Gauci CL: Effects of cloned interferon α_2 in normal volunteers: Febrile reactions and changes in circulating corticosteroids and trace metals. Antimicrob Agents Chemother 1983;23:589–592.

18 Horrocks PM, Jones AF, Ratcliffe WA, Holder G, White A, Holder R, Ratcliffe JG, London DR: Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. Clin Endocrinol 1990;32:127–134.

19 Partridge WM: Neuropeptides and the blood-brain barrier. Annu Rev Physiol 1983;45:73–82.

20 Smith RA, Norris F, Palmer D, Bernhardt L, Wills RJ: Distribution of alpha interferon in serum and cerebrospinal fluid after systemic administration. Clin Pharmacol Ther 1985;37:85–88.

21 Rohatiner AZS, Prior PF, Burton AC, Smith AT, Balkwill FR, Lister TA: Central nervous system toxicity of interferon. Br J Cancer 1983;47:419–422.

22 Blalock JE, Smith EM: Human leukocyte interferon: Structural and biological relatedness to adrenocorticotropic hormone and endorphins Proc Natl Acad Sci USA 1980;77:5972–5974.

23 White A, Smith H, Hoadley M, Dobson SH, Ratcliffe JG: Clinical evaluation of a two site immunoradiometric assay for adrenocorticotrophin in unextracted human plasma using monoclonal antibodies. Clin Endocrinol (Oxf) 1987;26:41–51.

24 Smith EM, Morrill AC, Meyer WJ III, Blalock JE: Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. Nature 1986;321:881–882.

25 Ferreira JA, Carstens ME, Taljaard JJF: Quantitative determination of lymphocyte $ACTH_{1-39}$. Neuropeptides 1990;15:11–15.

26 Woloski BMRNJ, Smith EM, Meyer WJ III, Fuller GM, Blalock JE: Corticotropin-releasing activity of monokines. Science 1985; 230:1035–1037.

27 McGillis JP, Hall NR, Goldstein AL: Thymosin fraction 5 (TF5) stimulates secretion of adrenocorticotropic hormone (ACTH) from cultured rat pituitaries. Life Sci 1988;42:2259–2268.

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EXHIBIT D

Original Paper

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Interferon-α Stimulates the Hypothalamic-Pituitary-Adrenal Axis in vivo and in vitro

Key Words
Interferon-α
CRH
ACTH
Cortisol

Abstract
The successful therapeutic use of interferon-α (IFN-α) in myeloproliferative disorders offered the possibility to test its acute and long-term effects on the hypothalamic-pituitary-adrenal (HPA) axis in humans. ACTH and cortisol plasma concentrations were measured in 8 patients hourly starting from 4 p.m. through 12 p.m. on three occasions. The first time all patients were studied before initiation of therapy, when the vehicle was injected alone. The patients were studied again on day 1 of IFN-α therapy (5 million units) and once more after 3 weeks of therapy. On the control day, plasma concentrations of ACTH and cortisol were in the range expected for this time of day. In contrast, after the first administration of IFN-α a significant stimulation of the HPA axis was observed. After 3 weeks of IFN-α therapy, no significant stimulation of the HPA axis occurred after administration of IFN-α. IFN-α-induced adaptive changes in the HPA axis were also indicated by a significantly enhanced ACTH and cortisol response to exogenously administered supramaximal doses of corticotropin-releasing hormone (CRH) when the patients had been on IFN-α treatment for 3 weeks. To determine the exact locus of the IFN-α action, in vitro experiments were performed using rat hypothalamic organ and primary pituitary and adrenal cell culture systems. Thereby a significant stimulation of hypothalamic CRH secretion and rat adrenal corticosterone production was observed after INF-α at concentrations of 5×10^{-8} *M* or 10^{-7} *M* respectively. In contrast, no direct IFN-α effect on pituitary ACTH secretion could be observed in vitro. It is concluded that IFN-α stimulates the HPA axis. The locus of action seems to be the hypothalamus, as well as the adrenal glands.

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Table 1. Characteristics of the patients treated with IFN-α

Pat. No.	Age	Sex	Diagnosis	Therapy
1	56	f	essential thrombocythemia	digoxin 0.1 mg spironolactone 50 mg butizide 5 mg
2	85	m	esential thrombocythemia	digitoxin 0.1 mg
3	66	f	polycythemia vera	digitoxin 0.1 mg
4	90	f	chronic myeloid leukemia	digoxin 0.1 mg dergocrinmesylate 1 mg
5	69	f	chronic myeloid leukemia	
6	46	f	essential thrombocythemia	
7	52	m	essential thrombocythemia	nifedipine 40 mg atenolol 100 mg chlorthalidone 25 mg
8	72	f	polycythemia vera	digoxin 0.1 mg spironolactone 50 mg butizide 5 mg piracetam 2,400 mg

Several years ago, direct interactions between the immune and neuroendocrine systems were first described [1, 2], and the existence of a lymphoid adrenal axis has been postulated [3]. Further studies have attempted to elucidate this relationship between the immune and neuroendocrine systems [4–9]. Concerning the signals directed from the immune to the neuroendocrine system, the soluble mediator substances of the immune system – the cytokines – seem to be of particular interest. Various cytokines – interleukin (IL)-1, IL-2 and IL-6, as well as interferon (IFN)-α and IFN-γ – have been reported to activate the hypothalamic-pituitary-adrenal (HPA) axis [10–21]. The therapeutic use of IFN-α [22] in a variety of diseases offered the opportunity to test its effect in vivo in humans.

This study evaluated the effects of both acute and chronic IFN-α administration on the HPA axis in humans. In addition, the effect of IFN-α was tested in vitro in hypothalamic organ and in primary pituitary and adrenal cell culture systems.

Materials and Methods

In vivo Study

Eight patients with myeloproliferative disorders (table 1), who received IFN-α for therapeutic reasons [22], were studied on three separate occasions after informed consent was obtained. On day 1, patients received placebo; on day 2 the patients were injected recombinant human IFN-α (5 million units subcutaneously) (Berofor; Boehringer Ingelheim, FRG); and finally the patients were studied again after they had been on therapy for 3 weeks (5 × 5 million units/week). To minimize the effect of diurnal variation of ACTH and cortisol concentrations, all tests were started at 3.30 p.m. IFN-α or placebo was injected at 4 p.m. Blood was collected everey 30 min until 5 p.m. and at hourly intervals thereafter until 12 p.m. In addition, a corticotropin-releasing hormone (CRH) test was performed in the same patients before starting the treatment and after 3 weeks of treatment. These tests were done 2–3 days prior to the 8-hour study described above, and the patients did not receive IFN-α on this day. All subjects were given 100 μg of the hypothalamic-releasing hormone for ACTH, i.e. CRH, in an intravenous bolus injection. For the benefit of a quiescent HPA axis, these tests were performed at 6 p.m. Blood was collected in prechilled EDTA tubes, immediately placed on ice and centrifuged within 2 h. Plasma was then separated and stored at –20 °C until assayed.

IFN-α plasma levels were determined using a cytopathic effect inhibition assay [23]. Test plasma were assayed for their ability to protect human A549 lung cancer cells from cytopathic effect induced by mouse encephalomyocarditis virus (EMC). In brief: the test cell line was incubated in microtiter plates, and 2 or 3 days later they were treated with plasma samples or IFN overnight before challenge with EMC. The cytopathic effect after 2 days was scored under a microscope. IFN-α2c (specific activity 320 IU/ng), laboratory standard HS12, calibrated to the international reference preparation G023-901-527 was used as positive control. The investigations were carried out in duplicate, the detection limit was 4 IU/ml. ACTH was measured by a commercially available IRMA (Euro-Diagnostics BV, Apeldoorn, The Netherlands), cortisol by a RIA (Baxter, USA). Body temperature was recorded whenever blood was drawn.

In vitro Studies

Male Sprague-Dawley rats weighing 200–250 g were sacrificed after CO_2 analgesia by decapitation. The hypothalamus, the pituitary gland and the adrenal glands were then removed for the respective organ or cell cultures.

Hypothalamic Organ Cultures. The hypothalamic region was removed with sterile scissors between the posterior border of the optic chiasma, the anterior border of the mammillary bodies and the lateral hypothalamic sulci as described previously [24]. Immediately after dissection, whole hypothalami were placed in 24-well plates (Costar, Cambridge, Mass., USA) at 37 °C in an atmosphere containing 5% CO_2. Each well contained 2 ml of Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK), supplemented with 2% fetal calf serum (FCS) (Gibco) and antibiotics (Gibco). After 2 h preincubation, the hypothalami were moved from well to well at 20-min intervals. The first three wells consisted of medium alone, the next two wells of medium with varying concentrations of IFN-α or plain medium as a negative control, the next two wells again of medium alone, and the last

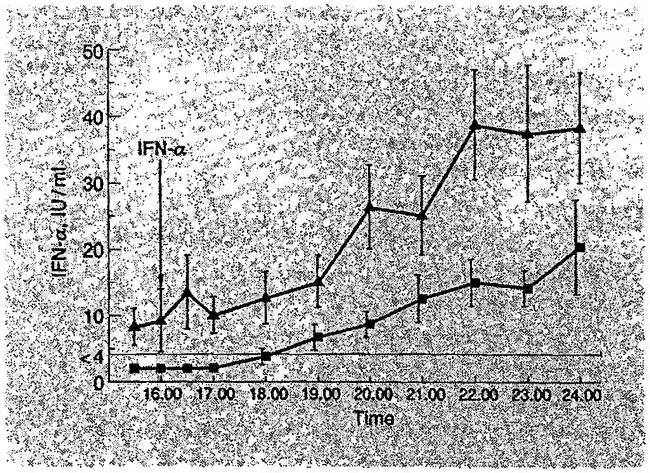

Fig. 1. Mean (± SEM) plasma IFN-α concentrations in patients with myeloproliferative disorders (■ = on day of first subcutaneous application of 5 million units IFN-α, ▲ = after 3 weeks of IFN-α therapy, 5 × 5 million units/week). The line across the bottom at 4 IU/ml IFN-α indicates the limit of detection of the assay used.

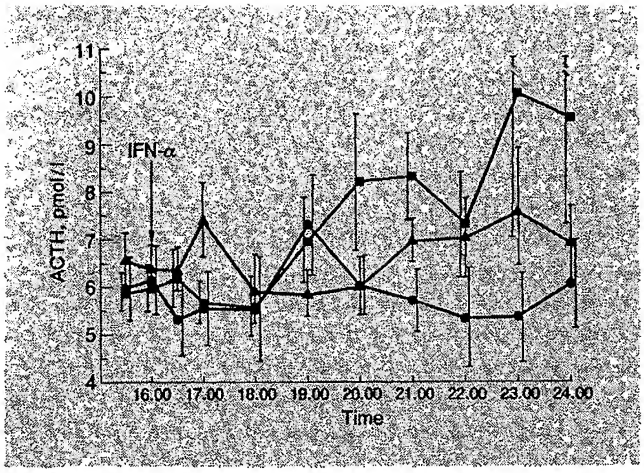

Fig. 2. Mean (± SEM) plasma ACTH concentrations in patients with myeloproliferative disorders (● = before IFN-α therapy, ■ = on day of the first subcutaneous application of 5 million units IFN-α, ▲ = after 3 weeks of IFN-α therapy 5 × 5 million units/week).

well of medium with 60 m*M* KCl for depolarization of the membrane to serve as positive control. Twelve hypothalami were used for each concentration, and hypothalami that failed to respond to KCl with at least a 70% increase of CRH release compared to basal release were excluded from analysis.

Pituitary Cell Culture. After dissection of the posterior and neurointermediate lobes, the anterior lobe of the pituitary gland was mechanically and enzymatically dispersed as described previously [24]. In short, the anterior lobes were first minced in Petri dishes and subsequently dispersed by a 20-min incubation with 0.1% collagenase type 4 (Sigma, St. Louis, Mo., USA) in DMEM. The pellets were washed twice by suspension and centrifugation at 160 *g* and resuspended in DMEM supplemented with 10% FCS. Cell yield was approximately 2×10^6 cells/gland and cell viability determined by trypan blue exclusion was always >95%. The cells were incubated for 4 days in a 24-well plate at a density of 5×10^5 cells/ml in DMEM with 10% FCS, 1% nonessential amino acids (Gibco) and antibiotics at 37 °C under 5% CO_2. The cells were then washed 3 times and incubated for 3 h with DMEM with or without IFN-α at concentrations ranging from 10^{-11} to 10^{-7} *M* in triplicate at 37 °C in an atmosphere of 5% CO_2. rCRH at a concentration of 10^{-7} *M* served as positive control. After incubation, the 24-well plate was centrifuged and the supernatant was collected and stored at –20 °C until determination of ACTH.

Adrenal Cell Culture. Adrenal glands were treated as described above for the pituitary gland. ACTH (Ciba, Basel, Switzerland) at a concentration of 10^{-7} *M* served as positive control for adrenal corticosterone release. Supernatants were stored at –20 °C until corticosterone was determined by RIA (ICN Biomedicals, Costa Mesa, Calif., USA).

Statistical Analysis

All results are expressed as the mean ± SEM. To evaluate a hormone response in vivo, the area under the curve was calculated by integration of the hormone levels in conventional units and time of testing in minutes. Comparing ACTH and cortisol plasma concentrations in vivo as well as body temperatue (fig. 2–4), the statistical evaluation was done using one-way analysis of variance, because some patients did not have complete values for the 3 days of investigation. The statistical analysis of CRH test was done as before comparing the area under the curve using paired t test. To evaluate a hormone response in vitro, mean increases of the respective hormone release over baseline secretion at varying IFN-α concentrations were compared by the Kruskal-Wallis test. Significance was accepted at $p \leq 0.05$.

Results

In vivo Study (fig. 1–4)

IFN-α plasma levels on day 1 of IFN-α treatment, as shown in figure 1, increased after 3 h and reached the maximum after 6 h. The curve of IFN-α plasma concentrations after 3 weeks of IFN-α treatment ran parallel, but shifted to a higher level. Recombinant human IFN-α at a dose of 5 million units induced an increase of cortisol plasma levels (fig. 3) and led to an elevation of the body temperature (fig. 4). Compared to their normal pattern

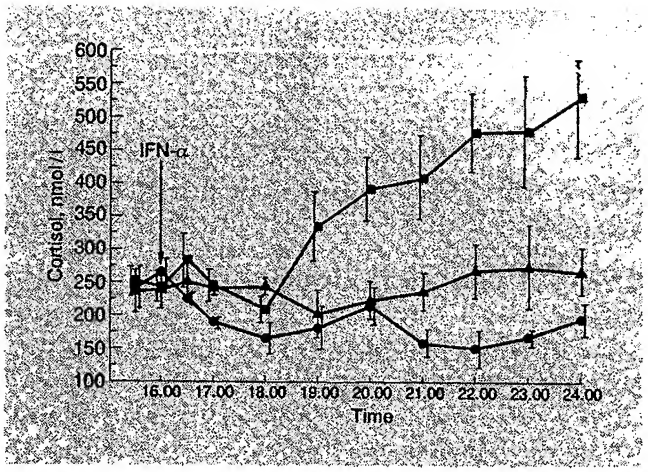

Fig. 3. Mean (± SEM) plasma cortisol concentrations in patients with myeloproliferative disorders (symbols are the same as in figure 2).

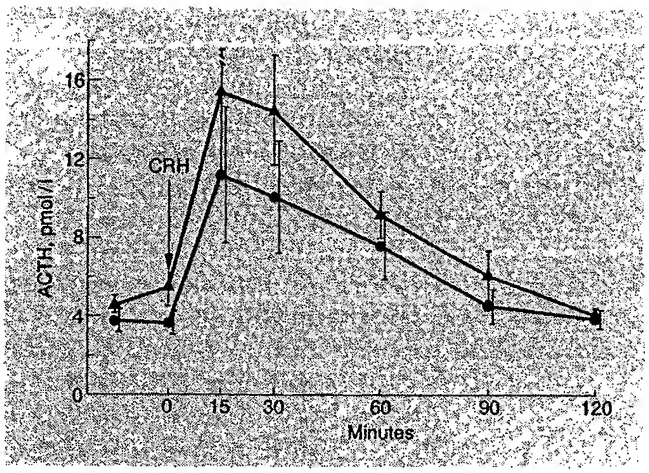

Fig. 5. Mean (± SEM) ACTH concentrations in patients with myeloproliferative disorders, before and after intravenous application of 100 μg CRH (● = before therapy with IFN-α, ▲ = after 3 weeks of subcutaneous therapy with 5 × 5 million units IFN-α/week).

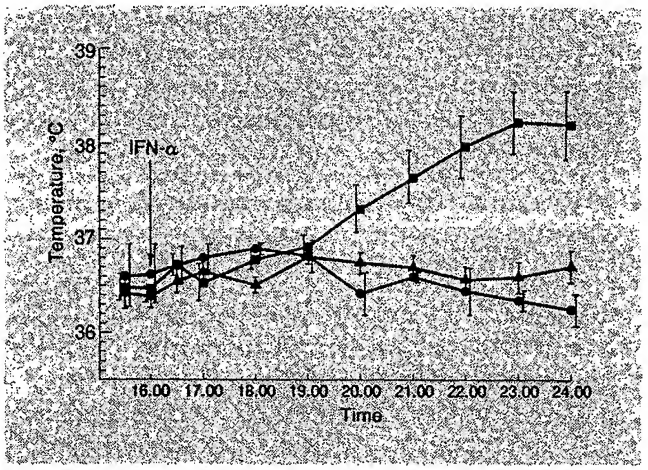

Fig. 4. Mean (± SEM) of axillary body temperature (°C) in patients with myeloproliferative disorders (symbols are the same as in figure 2).

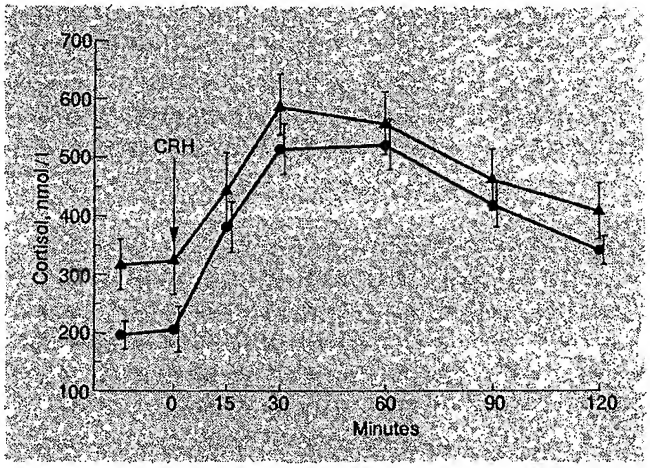

Fig. 6. Mean (± SEM) cortisol concentrations in patients with myeloproliferative disorders before and after intravenous application of 100 μg CRH (symbols are the same as in figure 5).

observed between 4 and 12 p.m. on the baseline day, when the patients received no IFN-α, ACTH plasma concentrations did not increase significantly (fig. 2) whereas cortisol plasma levels increased markedly, as expressed by a significant increase of the area under the curve ($p < 0.01$; fig. 3) after the first injection of IFN-α. The onset of this IFN effect was seen after 3 h and plasma cortisol concentrations remained elevated thereafter until the end of the observation period of 8 h and thus paralleled the plasma concentrations of IFN-α shown in figure 1. When the patients were studied again after they had been on IFN-α for 3 weeks, only slightly but not significantly elevated ACTH and cortisol plasma levels compared to the baseline day could be observed. Body temperature increased after the first injection, which is also expressed by a significant increase of the area under the curve ($p < 0.05$; fig. 4) and returned to the baseline level after 3 weeks of IFN-α treatment.

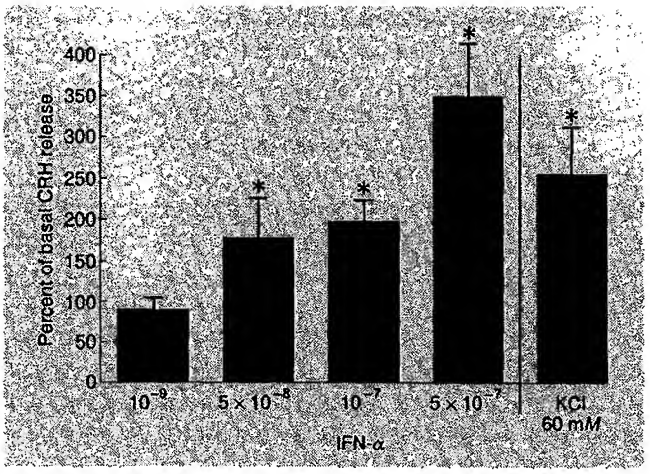

Fig. 7. Effect of graded IFN-α concentrations on the CRH secretion in primary rat hypothalamic organ cultures expressed as percent of control. KCl (60 m*M*) for depolarization of the cell membrane served as a positive control (*p < 0.05 compared to basal CRH release).

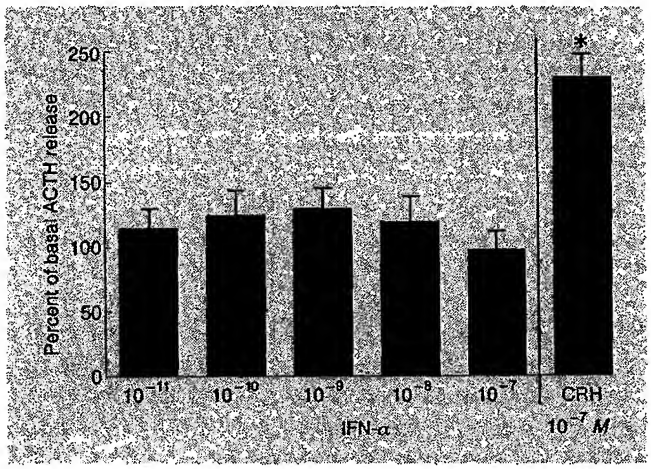

Fig. 8. Effect of graded IFN-α concentrations on the ACTH secretion in primary rat pituitary cell cultures expressed as percent of control. CRH (10^{-7} *M*) served as a positive control (*p < 0.05).

CRH Test (fig. 5, 6)

Despite the return of body temperature to the baseline level after 3 weeks of IFN-α, the area under the curve of ACTH plasma concentrations was significantly greater ($p < 0.01$) than on the baseline day. This significant difference ($p < 0.05$) could also be observed in the area under the curve of the plasma cortisol levels.

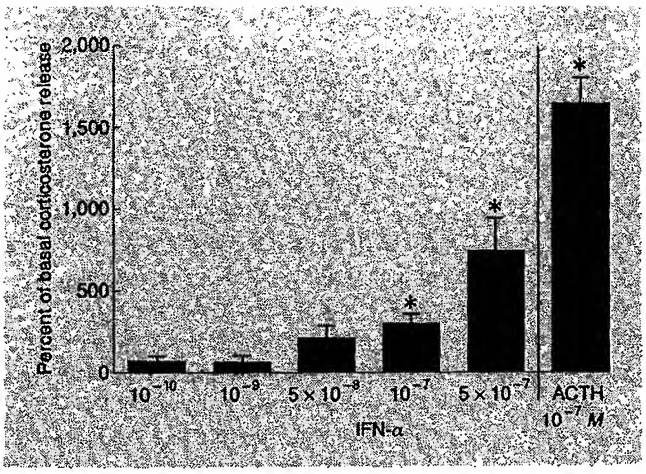

Fig. 9. Effect of graded IFN-α concentrations on the corticosterone secretion in primary rat adrenal cell cultures expressed as percent of control. ACTH (10^{-7}*M*) served as a positive control (*p < 0.05).

In vitro Studies

In the primary rat hypothalamic organ cultures, a significant dose-dependent increase of CRH release could be observed starting at IFN-α concentrations of 5×10^{-8}*M* (fig. 7). In the primary dispersed pituitary cell cultures, no effect of IFN-α on ACTH release could be registered over an IFN-α concentration range from 10^{-11} to 10^{-7} *M* (fig. 8). In primary dispersed adrenal cells, IFN-α at a concentration of 10^{-7} *M* induced a significant increase of corticosterone release, which was still higher at 5×10^{-7} *M* IFN-α (fig. 9).

Discussion

This study demonstrates that IFN-α stimulates the HPA axis in vivo and in vitro. When patients received IFN-α for the first time a significant increase in plasma cortisol concentrations and body temperature was observed. Chronic subcutaneous application of IFN-α led to adaptive changes such that the cortisol response to IFN-α was clearly diminished. Furthermore, in the CRH test, where supramaximal doses of the hypothalamic releasing peptide are administered, increased ACTH and cortisol responses could be registered after 3 weeks of IFN-α therapy. A direct effect of IFN-α on the hypothalamus and the adrenal gland described here for the first time might be responsible for this activation of the HPA axis.

Since the rise of cortisol following administration of IFN-α is not as abrupt as after other known activators of the HPA axis, such as CRH, one might argue that the stimulation is either unspecific, e.g. caused by IFN-α-induced fever or through other mediators. Holsboer et al. [19], for instance, interpreted their observation of IFN-α-induced stimulation of the HPA axis in this way. A favorite candidate for a mediator substance involved in the mediation of the IFN-α effects would be the generation of other ILs by IFN-α, such as IL-1 and IL-6, which have both a pyrogenic and ACTH-releasing activity. Although elevation of body temperature as cause for the activation of the HPA axis cannot be totally ruled out, several points argue against this possible mechanism of action. First, in vitro a direct action of IFN-α on the hypothalamus and adrenal gland has been observed, and second, after chronic IFN-α administration the rise in temperature was abolished whereas the HPA axis was still activated, as expressed by the findings of the CRH test. Furthermore, a specific activation of the HPA axis through stimulation of hypothalamic CRH release unrelated to its pyrogenic effect has also been proposed for another cytokine, namely IL-1 [16]. In addition, the time course of the ACTH and cortisol curves followed the course of IFN-α plasma concentrations after subcutaneous injection.

The chronic activation of the HPA axis by IFN-α is reminiscent of the alterations seen in anorexia nervosa, depression, alcoholism and compulsive running [25], and could also be responsible for the neuropsychiatric disorders previously described in patients receiving treatment with IFN-α [26].

The previously reported inhibitory effect of IFN-α on insulin [27] and sex steroid production [28] in conjunction with the activation of the HPA axis would fit well into Selye's [29] concept of the stress reaction and the general adaptation syndrome. In order to optimally oppose a stressor, e.g. a severe infection, the organism, by elevation of IFN-α plasma concentrations, would activate endocrine systems like the HPA axis that has powerful desirable effects on the cardiovascular system and metabolism, and deactivate systems like the sex steroids, that are not necessary in the acute stress reaction. Thus, the immune system with its mediator substances – the cytokines – would function as a sensory organ as proposed previously by Blalock [5], by stimulating or inhibiting the various endocrine systems. In turn, in situations of prolonged elevation of IFN-α, when the initial stressor persists, glucocorticoids will act upon the immune system to prohibit exaggerated and self-destroying activiy of the immune system.

In summary, IFN-α must be added to the ever-growing list of substances with stimulating effects on the HPA axis. Adding to the results of other studies, this effect was demonstrated in vivo and in vito. It should be noted that only pharmacological doses of IFN-α were effective in vivo, so that any physiological role of IFN-α in the regulation of the HPA axis remains speculative.

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References

1 Lymangrover JR, Brodish A: Tissue corticotrophin-releasing factor (CRF): An extra-hypothalamic CRF in the peripheral blood of stressed rats. Neuroendocrinology 1973;12: 225–235.

2 Pierpaoli W, Sorkin E: Relationship between thymus and hypophysis. Nature 1967;215: 834–837.

3 Smith EM, Meyer WJ, Blalock JE: Virus induced corticosterone in hypophysectomized mice: A possible lymphoid adrenal axis. Science 1982;218:1311–1312.

4 Shavit Y, Lewis JW, Terman GW, Gale RP, Liebeskind JC: Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science 1984;223:188–190.

5 Blalock JW: The immune system as a sensory organ. J Immunol 1984;132:1067–1070.

6 Blalock JW, Bost KL, Smith EM: Neuroendocrine peptide hormones and their receptors in the immune system. J Neuroimmunol 1985; 10:31–40.

7 Westly HJ, Kleiss AJ, Kelley KW, Wong PKY, Yuen PH: Newcastle disease virus-infected splenocytes express the proopiomelanocortin gene. J Exp Med 1986;163:1589–1594.

8 Weigent DA, Blalock JE: Interactions between the neuroendocrine and immune systems: Common hormones and receptors. Immunol Rev 1987;100:78–108.

9 Breder CD, Dinarello CA, Saper CB: Interleukin-1 immunoreactive innervation of the human hypothalamus. Science 1988;240: 321–324.

10 Scott GM, Ward RJ, Wright DJ, Robinson JA, Onwubalili JK, Gauci CL: Effects of cloned interferon-α_2 in normal volunteers: Febrile reactions and changes in circulating corticosteroids and trace metals. Antimicrob Agents Chemother 1983;23:589–592.

11 Woloski BMRNJ, Smith EM, Meyer WJ III, Fuller GM, Blalock JE: Corticotropin-releasing activity of monokines. Science 1985; 230:1035–1037.

12 Besedovsky H, Del Rey A, Sorkin E, Dinarello CA: Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science 1986;233:652–654.

13 Roosth J, Pollard RB, Brown SL, Meyer WJ: Cortisol stimulation by recombinant interferon-α_2. J Neuroimmunol 1986;12:311–316.

14 Calogero AE, Luger A, Gallucci WT, Gold PW, Chrousos GP: Interleukin-1 and interleukin-2 stimulate hypothalamic corticotropin-releasing hormone but not pituitary ACTH secretion in vitro. Proc 69th Annu Meet Endocrine Society, Indianapolis 1987, abstr 1001, p 271.

15 Berkenbosch F, Van Oers J, Del Rey A, Tilders F, Besedovsky H: Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. Science 1987;238: 524–526.

16 Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W: Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 1987;238:522–524.

17 Bernton EW, Beach JE, Holaday JW, Smallridge RC, Fein HG: Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. Science 1987;238:519–521.

18 Brown SL, Smith LR, Blalock JE: Interleukin-1 and interleukin-2 enhance proopiomelanocortin gene expression in pituitary cells. J Immunol 1987;139:3181–3183.

19 Holsboer F, Stalla GK, Von Bardeleben U, Hammann K, Müller H, Müller OA: Acute adrenocortical stimulation by recombinant gamma interferon in human controls. Life Sci 1988;42:1–5.

20 Gonzalez MC, Riedel M, Rettori V, Yu WH, McCann SM: Effect of recombinant human gamma interferon on the release of anterior pituitary hormones. PNEI 1990;3:49–54.

21 Karanth S, McCann SM: Anterior pituitary hormone control by interleukin-2. Proc Natl Acad Sci USA 1991;88:2961–2965.

22 Merigan TC: Human interferon as a therapeutic agent. N Engl J Med 1988;318:1458–1460.

23 Kawade Y, Watanabe Y: Neutralization of interferon by antibody: Appraisals of methods of determining and expressing the neutralization titer. J Interferon Res 1984;4:571–584.

24 Luger A, Calogero AE, Kalogeras K, Gallucci WT, Gold PW, Loriaux DL, Chrousos GP: Interaction of epidermal growth factor with the hypothalamic-pituitary-adrenal axis: Potential physiologic relevance. J Clin Endocrinol Metab 1988;66:334–337.

25 Chrousos GP, Luger A, Avgerinos PC, Kling MA, Oldfield EH, Nieman LK, Schürmeyer T, Loriaux DL, Gold PW: Corticotropin-releasing hormone: Relevance to human physiology and pathophysiology; in d'Agata R, Chrousos GP (eds): Recent Advances in Adrenal Regulation and Function. New York, Raven Press, 1987, pp 121–135.

26 Adams F, Quesada JR, Gutterman JU: Neuropsychiatric manifestations of human leucocyte interferon therapy in patients with cancer. J Am Med Assoc 1984;252:938–941.

27 Shimizu F, Shimizu M, Kamiyama K: Inhibitory effect of interferon on the production of insulin. Endocrinology 1985;117:2081–2084.

28 Kauppila A, Cantell K, Jänne O, Kokko E, Vihko R: Serum sex steroid and peptide hormone concentrations, and endometrial estrogen and progestin receptor levels during administration of human leucocyte interferon. Int J Cancer 1982;29:291–294.

29 Selye H: The general adaption syndrome and the diseases of adaption. Clin Endocrinol 1946;6:117–216.

EXHIBIT E

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CASE REPORT

An interferon-α-induced psychotic disorder in a patient with chronic hepatitis C

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The neuropsychiatric complications of interferon-α (INF-α) treatments, as major adverse consequences of therapeutic cytokine administration, have primarily been described in the early 1980s, with the onset of interferon's therapeutic use for some malignant neoplasm and viral infections [3]. There is a considerable amount of literature on the neuropsychiatric side effects associated with INF-α, referring to depression (more frequently), personality changes, and impaired cognition; less often to psychosis and delirium; and rarely to mania [6, 8, 11]. Neurotoxicity seems to be mediated through neuroendocrine and neurotransmitter mechanisms, cytocines, and free radicals [7]. It seems that the knowledge of this kind of toxicity gives opportunities for a better understanding of the pathophysiological mechanisms of several diseases, especially while there is an increased interest concerning the possible implication of cytokines in depression [2, 9]. However, severe psychiatric manifestations represent the most important reason for the discontinuation of INF-α [11]. Adverse psychiatric effects are related to dose, duration of therapy, and route of administration [6]; they are usually reversible, though sometimes may be persistent [11].

In this paper we present the case of induced INF-α2b psychotic symptoms in a man with chronic hepatitis C one day after the administation of his standard dose of INF-α2b.

CASE REPORT

A 29-year-old unmarried man was admitted to our clinic because of delusional ideas of persecution, auditory hallucinations, insomnia and psychomotor agitation. One day before admission he received his standard dose of 6 million units of INF-α2b subcutaneously. One year before and after a routine blood test, he was diagnosed for hepatitis C. The histopathological findings of a liver biopsy were suggestive of chronic hepatitis (infiltration of inflammatory cells, especially leukocytes, as well as areas with bridging necrosis). A month later he started receiving 6 million INF-α2b 3 times a week.

Cannabis abuse is reported since he was 15 years old, with heroin dependence 5 years later. The diagnosis of his infectious disease motivated him to stop using illicit drugs. He has few close friends, does not seem to enjoy emotional ties or intimate relations and spends many hours in solitary activities. According to his family's description, he is a very introverted person and never talks about his problems. The patient has a burdened psychiatric family history. His grandfather was diagnosed as schizophrenic as well as one of his first cousins.

On admission aspartate aminotransferase was 55 U/L (normal range: 0–45 U/L). Other laboratory tests were normal (complete blood cell count, glucose, urea, creatinine, alanine aminotransferase, γ-glutamyl transaminase, total bilirubin, alkaline phosphatase, cholesterol,

Correspondence and reprints.

triglyceridia, and urine analysis). The neurologic examination was normal as well.

The patient was treated with 10 mg haloperidol im bid replaced by risperidone 3 mg bid after 3 days. Five days after admission, he had a full remission of his symptoms with a complete return to a premorbid level of functioning. The patient was discharged from hospital 10 days later, receiving risperidone 2 mg bid. He did not show any symptoms during 5 months of follow-up. INF-α2b was not reintroduced because of its association with the appearance of psychotic symptoms.

DISCUSSION

Early side effects of an INF-α treatment include a flu-like syndrome that emerges with the start of INF-α injections, whereas neuropsychiatric manifestations appear after several weeks of therapy [11]. The patient described above was receiving high doses of INF-α [10], and psychotic symptoms appeared after 11 months of continuous treatment. However, the proposition of dopamine depletion, mediated through its binding to opiate receptors, as a mechanism of INF-α-induced neurotoxicity [6] even led to clinical trials in schizophrenic patients, with inconsistent results [1, 4]. In accordance with the literature, the patient's recovery occurred a few days after the use of neuroleptics and discontinuation of INF-α2b.

Our case has some analogy to a case presented by Lemonnier et al. [5], although their patient had no psychiatric antecedent. On the other hand, our patient had a history of addictive behavior and a burdened psychiatric family history. Unfortunately, there are no clear predictive factors for the development of neuropsychiatric complications of INF-α treatments, though a previous history of psychiatric disorder, brain dysfunction or addictive behavior is usually considered as a potential contraindication for INF-α treatment [11]. This factor has not been taken into consideration in our patient's case.

REFERENCES

1 Cabrera Gomez JA, Cordero Gutierrez JR, Fernandez Lopez O, Reyes Gutierrez B, Romero Garcia K, Simon Conuegra J, et al. Treatment of schizophrenic disorder, paranoid type, with intramuscular recombinant alpha-2b interferon. Biotherapy 1993 ; 7 : 27-37.

2 Danzer R, Wollman E, Vitkovic L, Yirmiya R. Cytokines and depression: fortuitous or causative association? Mol Psychiatry 1999 ; 4 : 328-32.

3 Hadden JW. Immunopharmacology of cancer, infection and autoimmunity. Fundam Clin Pharmacol 1987 ; 4 : 283-96.

4 Katila H, Cantell C, Appelberg B, Wahlbeck K, Naukkarinen H, Rimon R. Interferon-alpha as adjuvant treatment in chronic schizophrenia. Neuropsychobiology 1993 ; 28 : 192-6.

5 Lemonnier E, Condat B, Paillere-Martinot ML, Chollet R, Allilaire JF. Syndrome de perseceution sous interferon: à propose d'un cas. Ann Med Psychol 1996 ; 154 : 246-8.

6 Lerner DM, Stoudemire A, Rosenstein DL. Neuropsychiatric toxocity associated with cytokine therapies. Psychosomatics 1999 ; 40 : 428-35.

7 Licinio L, Kling MA, Hauser P. Cytokines and brain function: relevance to interferon-alpha-induced mood and cognitive changes. Semin Oncol 1998 ; 25 (Suppl 1) : 30-8.

8 Monji A, Yoshida I, Tashiro KI, Hayashi Y, Tashiro N. A case of persistent manic depressive illness induced by interferon-alpha in the treatment of chronic hepatitis C. Psychosomatics 1998 ; 39 : 562-4.

9 Okada F. Interferon-induced depression: just one of Bonhoefer's exogene reactiontypen or a clue to understanding psychoimmunological aspects of depression? J Mol Med 1995 ; 73 : 99-100.

10 Shiffman ML. The use of high-dose interferon in the treatment of chronic hepatitis C. Semin Liver Dis 1999 ; 19 (Suppl 1) : 25-34.

11 Vial T, Choquet-Kastylevsky G, Liautard C, Descotes J. Endocrine and neurological adverse effects of therapeutic interferons. Toxicology 2000 ; 142 : 161-72.

EXHIBIT F

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CASE REPORT

Psychosis in a methadone-substituted patient during interferon-alpha treatment of hepatitis C

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Abstract

Interferon-alpha (IFN-α) is the only effective treatment for chronic hepatitis B and C. Over 2/3 of methadone-substituted patients suffer from chronic hepatitis C but a history of psychiatric disorders or drug addiction is still seen as a contraindication for IFN-α because of a possible increased risk of severe psychiatric side effects such as depression, suicide attempts or psychotic episodes. We report on the case of a 33-year-old patient with chronic hepatitis C and a positive psychiatric history (drug abuse, borderline personality and four suicide attempts). After 4 months of therapy with IFN-α he developed a psychosis with persecution mania, complex thought disorder, disturbance of sexual identity, sleeplessness, anxiety, depression and increased irritability with suicidal thoughts. Symptoms did not disappear after discontinuation of interferon treatment. To our knowledge, there are no other reports of persistent psychosis with a possible association to interferon treatment. Development of psychosis and other psychiatric side-effects may be an indication of possible neuromodulatory effects of IFN-α with long-term treatment. On the other hand, the treatment for hepatitis C was successful. Ideas for safer treatment in methadone patients with psychiatric co-morbidity and chronic hepatitis C are needed.

Introduction

Interferon alpha (IFN-α) is the only effective treatment for chronic hepatitis B and C (Cirelli & Tyring, 1995; Haria & Benfield, 1995). However, in patients with drug abuse or methadone substitution the treatment with IFN-α is contraindicated, because further intravenous drug abuse may lead to re-infection. Additionally the high rate of psychiatric co-morbidity with increased risk of severe psychiatric side effects, such as development of depression, mania, increased irritability, changes in personality, hallucinations or delirium might complicate the treatment further (Renault & Hoofnagle, 1989;

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Muraoka *et al.*, 1996). Even sudden unexpected suicide attempts while on IFN-α have been reported, but most of them occurred in patients without psychiatric disorders (Janssen *et al.*, 1994; Gaudin *et al.*, 1995). Currently, only a few reports have tried to find risk-factors for these side-effects (Renault *et al.*, 1987; Renault & Hoofnagle, 1989; Janssen *et al.*, 1994). McDonald, Mann & Thomas (1987) identified a higher risk of patients with hepatitis and AIDS developing psychiatric side-effects from IFN-α. For patients with psychiatric co-morbidities recent studies could not confirm an increased risk. Cabrera-Gomez *et al.* (1994) treated chronic paranoid schizophrenic patients with antipsychotics and IFN-α-2b in a placebo-controlled study and patients with IFN-α had a better outcome and needed lower doses of antipsychotics than the group without. Van Thiel *et al.* (1995) have successfully treated patients with hepatitis C and drug addiction, affective or psychotic disorders in cooperation with their psychiatrists. Recently, it has also been shown that patients with hepatitis C and mood or anxiety disorders, thought of as relatively contraindicated for treatment with IFN-α, did not develop more psychiatric side-effects compared with normal controls (Pariante *et al.*, 1999). The authors found no evidence that psychiatric cases were more likely to stop IFN-α therapy than controls. Furthermore, even in a putative case of depression as a side-effect of IFN-α, effective treatment possibilities were evident (Levenson & Fallon, 1993).

Renault *et al.* (1987) described three forms of psychiatric side effects in long-term treatment with IFN-α; 17% developed organic syndromes, called organic personality syndrome, characterized by irritability and short temper, organic affective syndrome with emotional weakness, depression, fearfulness and delirium, marked by clouding of consciousness, agitation, paranoia and suicidal potential. There are a few case reports of psychotic episodes in drug users during IFN-α treatment (McDonald *et al.*, 1987; Renault *et al.*, 1987; Hendrik, 1994; Muraoka *et al.*, 1996) and they have been interpreted as flash-back psychosis, or delirium, because of earlier drug abuse. In all cases psychiatric side-effects stopped after dose reduction or discontinuation of interferon treatment.

We report on a patient in a methadone substitution programme with a positive psychiatric history and chronic hepatitis C who developed severe psychotic symptoms during therapy with IFN-α, which persisted after discontinuation of treatment.

Case report

A 33-year-old student with a history of polydrug abuse and a borderline personality disorder was treated at our hospital in 1997. The student reported continuous abuse of cannabis and LSD since he was 14 years old. In the next 2 years he used illegal drugs and developed addiction to intravenous heroin with additional abuse of cannabis, alcohol, benzodiazepines, LSD and ecstasy. In 1992 he was taken into a methadone substitution programme. During this time he occasionally used cannabis, LSD or ecstasy but there was no history of psychotic episodes. Between 1993 and 1996 he made three suicide attempts involving concurrent use of benzodiazepines and alcohol, apparently caused by difficulties in his relationships with girlfriends. Therefore, he was treated twice in a psychiatric hospital with the diagnosis of short depressive episode and borderline personality disorder. Again, there were no signs of a developing paranoid psychosis.

He was diagnosed with hepatitis C in 1989. The first liver biopsy showed a chronic active hepatitis. Four years later a second liver biopsy followed, without therapeutic consequences. In 1995, during methadone substitution, the liver biopsy was repeated and confirmed nascent fibrosis. Treatment was denied because of the psychiatric and drug history of the patient. Nevertheless, in 1996 he found a hepatologist who began treatment with IFN-α-2a (3×5 mu subcutaneous per week). During this time, methadone treatment was continued with regular urinalysis for traces of drug use.

Side effects during interferon treatment

During the first 3 weeks of the treatment he complained about an 'influenza-like' syndrome, increased tiredness, sleepiness, concentration difficulties and loss of interest, which improved during the following weeks. After 5 months the hepatologist noticed for the first time some psychopathological changes in the form of paranoia and anxiety. After 6 months he developed a complex symptomatology with loss of appetite and weight, sleepiness, anxiety, depression and

increased irritability. After 8 months his symptoms changed to delusions of persecution, suspiciousness, fear of being poisoned, loss of sexual identification, rapid changes in mood (depressive to dysphoric or aggressive behaviour) and social withdrawal with growing suicidal thoughts. Because there was no change under neuroleptic treatment with sulpirid, the patient was admitted to our hospital. When we saw the patient, he showed complex difficulties in formal thoughts. As well as the symptoms reported above, the patient was sometimes also disorientated with an inability to act logically.

Physical examination showed no major abnormalities. Blood parameters were normal, especially liver enzymes. Urinalysis was only positive for methadone and the treating physician confirmed that urinalysis was negative in the last 12 months (besides methadone). ECG, EEG and cCT were normal.

Therapy

The symptoms were interpreted as an organic psychotic episode, possibly associated with IFN-α, but the symptoms did not disappear after discontinuation of interferon treatment. Because of his paranoid, aggressive and suicidal behaviour, the patient had to be treated with antipsychotic medication (haloperidol and melperone) over 3 months. The methadone substitution was continued. Treatment was successful, but the symptoms returned after reduction of antipsychotics so that he had to take olanzapine as continuation treatment. With additional psychotherapeutic and social help, he was able to leave hospital with a reduced dose of methadone. Treatment of hepatitis was also successful, with normalization of liver parameters and a negative PCR (polymerase chain reaction for detection of viral RNA) which remained negative for 6 months as a criterion for a sustained response.

Discussion

Because of the patient's psychiatric history and ongoing methadone treatment, nearly 8 years and three liver biopsies were necessary before treatment with IFN-α was begun. However, the negative PCR result and normalized transaminases showed that the treatment was successful. From our experience this case showed typical development of side-effects caused by IFN-α: in the first 3 weeks there was a dominance of sleepiness, asthenia and concentration difficulties, disappearing after the fourth week. Severe psychiatric side effects occurred for the first time after 4 months of therapy. During months 5 and 6 symptoms could be interpreted as a mixture of organically induced deliriant and affective syndrome, combined with psychotic symptoms. Unlike other case reports, the symptoms in this case did not disappear after discontinuation of interferon treatment, an effect comparable to the concept of drug-induced paranoid psychosis in a patient with a hypothetical increased vulnerability for schizophrenia after long-term abuse of different illegal drugs. Our patient can be considered as being a 'high-risk patient' for developing psychiatric side-effects during IFN-α treatment. However, even if patients in methadone maintenance programmes often show psychiatric co-morbidities (Rounsaville *et al.*, 1982), they do not necessarily develop manifest psychotic episodes for months. Until the therapy with IFN-α was begun in our patient, there was no sign of any psychosis. Because of the lack of recent illicit drug abuse during methadone substitution, psychotic episodes cannot readily be explained as 'illegal-drug-induced' or 'flash-back' psychosis. On the other hand, from this single case we cannot conclude a direct causal relationship between IFN-α treatment and the development of psychosis. Mechanisms for the psychiatric side effects of IFN-α remain unknown. Neurotoxicity may play an important role during the first 3 weeks (Merimsky & Chaitchik, 1992; Gutterman, 1994), but possibly not for the organic syndromes after several months of treatment. There are differences in the quality and severity of early psychiatric side effects and later side-effects. Personality changes and suicide attempts are mostly reported after the third or fourth month or later with complex psychiatric changes in patients (Adams, Quesada & Gutterman, 1984; Janssen *et al.*, 1994), indicating possible neuromodulatorys effects of long-term interferon treatment. In fact, there is some evidence of an influence of IFN-α on NMDA responses through opioid receptors (Katafuchi, Take & Hori, 1995). Glutamate, acting as an excitatory amino acid (EAA) on NMDA receptors, is known to play an important role in regulating synaptogenesis and neuronal development in the central nervous system with neurotoxicity in high doses

(Meldrum & Garthwaite, 1990). Furthermore, it is posited as being involved in the pathogenesis of schizophrenia and addiction (Heresco-Levi & Javitt, 1994). However, it remains unclear if IFN-α crosses the blood–brain barrier under some conditions, or if the neuropsychiatric changes may be explained by other indirect mediators such as cytokines (IL-1, TNF) or hormonal changes (Valentine *et al.*, 1998).

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References

ADAMS, F., QUESADA, J., GUTTERMAN, J. (1984) Neuropsychiatric manifestation of human leukocyte interferon therapy in patients with cancer, *Journal of the American Medical Association*, 252, 938–941.

CABRERA GOMEZ, J. A., GUTIERREZ, J. R., LOPEZ, O., GUITIEREZ, B., GARCIA, K., CONSUEGRA, J., CRUZ, R., QUEVEDO, A., CAPDEGILLE, I., FALCON, M., SERRANO, S., GALINDO, M., SAURA, P. & RIVERO, Y. (1994) Treatment of schizophrenic disorder, paranoid type, with intramuscular recombinant alpha-2b interferon, *Biotherapy*, 7, 27–37.

CIRELLI, R. & TYRING, S. K. (1995) Major therapeutic use of interferons, *Clinical Immunotherapeutics*, 3, 27–87.

GAUDIN, J. L., FAURE, P., GODINOT, H., GERARD, F. & TREPO, C. (1995) The French experience of treatment of chronic type D hepatitis with a 12-month course of interferon alpha-2B. Result of a randomized controlled trial, *Liver*, 15, 45–52.

GUTTERMAN, J. U. (1994) Cytokine therapeutics: lessons from interferon-alpha, *Proceedings of the National Academy of Science USA*, 91, 1198–1205.

HARIA, M. & BENFIELD, P. (1995) Interferon-alpha-2a, *Drugs*, 50, 873–896.

HENDRIK, A. (1994) Psychosis during interferon in eosinophilic leukemia, *Clinical and Laboratory Haematology*, 16, 295–296.

HERESCO-LEVI, U. & JAVITT, D. C. (1998) The role of N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission in the pathophysiology and therapeutics of psychiatric syndromes, *European Neuropsychopharmacology*, 8, 141–152.

JANSSEN, H. L. A., BROUWER, J. T., VAN DER MAST, R. C. & SCHALM, S. W. (1994) Suicide associated with alpha-interferon therapy for chronic viral hepatitis, *Journal of Hepatology*, 21, 241–243.

KATAFUCHI, T., TAKE, S. & HORI, T. (1995) Roles of cytokines in the neural immune interactions: modulation of NMDA-responses by IFN-alpha, *Neurobiology*, 3, 319–327.

LEVENSON, J. L. & FALLON, H. J. (1993) Fluoxetine treatment of depression caused by interferon-alpha, *American Journal of Gastroenterology*, 88, 760–761.

MCDONALD, E. M., MANN A. H. & THOMAS, H. C. (1987) Interferons as mediators of psychiatric morbidity, *Lancet*, 2, 1175–1178.

MELDRUM, B. & GARTHWAITE, J. (1990) Excitatory amino acid neurotoxicity and neurodegenerative disease, *Trends in Pharmacological Science*, 11, 379–387.

MERIMSKY, O. & CHAITCHIK, S. (1992) Neurotoxicity of interferon-alpha, *Anti-Cancer Drug*, 3, 567–570.

MURAOKA, H., SANEFUJI, T., KEIDA, R., TSUJI, R., ABE, H., UCHIMURA, Y., SATA, M., TANIKAWA, K., NOSE, I., UCHIMURA, N. & NAKAZAWA, Y. (1996) A patient with chronic hepatitis C and a history of abuse of analeptic drugs, who showed hallucination und delusion with interferon administration, *Kurume Medical Journal*, 43, 73–77.

PARIANTE, C. M., ORRU, M. G., BAITA, A., FARCI, M. G. & CARPNICELLO, B. (1999) Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders, *Lancet*, 10, 131–132.

RENAULT, P. F., HOOFNAGLE, J. H., PARK, Y., MULLEN, K. D., PETERS, M., JONES, B., RUSTGI, V. & JONES, A. (1987) Psychiatric complications of long-term interferon alpha therapy, *Archives of Internal Medicine*, 147, 1577–1580.

RENAULT, P. F. & HOOFNAGLE, J. H. (1989) Side effects of alpha interferon, *Seminars in Liver Disease* 9, 273–277.

ROUNSAVILLE, B. J., WEISSMANN, M. M., KLEBER, H. D. & WILBER, C. (1982) Heterogenity of psychiatric diagnosis in treated opiate addicts, *Archives of General Psychiatry*, 39, 161–166.

VALENTINE, A. D., MEYERS, C. A., KLING, M. A., RICHELSON, E. & HAUSER, P. (1998) Mood and cognitive side effects of interferon-alpha therapy, *Seminars in Oncology*, 25, 39–47.

VAN THIEL, D. H., FRIEDLANDER, L., MOLLOY, P. J., FAGIUOLI, S., KANIA, R. J. & CARACENI, P. (1995) Interferon-alpha can be successfully in patients with hepatitis C virus-positive chronic hepatitis who have psychiatric illness, *European Journal of Gastroenterology and Hepatology*, 7, 165–168.

EXHIBIT G

Interferon α – Induced Psychotic Disorder in a Patient with Chronic Hepatitis B

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Interferon has remained the mainstay for treating patients suffering from chronic viral hepatitis. However, its efficiency has been limited by the neuropsychiatric side effect profile that it carries; neurotransmitter alterations in the central nervous system (CNS) have been correlated to psychiatric complications of Interferon α. Although mood disorders such as depression occasionally develop during Interferon α therapy, psychotic disorders have been rarely reported. We present a case of Interferon α 2b induced psychotic symptoms in a young male with hepatitis B and review the relevant literature.

Key words: Interferon alpha, hepatitis, psychosis

INTRODUCTION

Hepatitis B has a worldwide distribution and is a major public health problem in developing countries where most people become infected with hepatitis B virus during childhood.[1,2] Around eight to ten percent of people in the general population become chronically infected.[3] The socio-economic burden of the disease on these countries is enormous. It is estimated that five to eight percent people in Pakistan are suffering from hepatitis B.[4]

Interferon has remained the mainstay of therapy for patients suffering from chronic viral hepatitis. However, its efficacy is limited by the neuropsychiatric side effect profile that it carries. It gives rise to well-documented syndromes including depression with suicidal ideation, personality changes and cognitive disturbances. Together these side-effects complicate interferon alpha therapy in 30 – 80 % of treated patients sometimes leading to cessation of therapy.[5] Modulation of opioid, serotonin, dopamine and glutaminergic neurotransmitter systems has been correlated to psychiatric complications of Interferon α.[6] Although mood disorders such as depression occasionally develop during Interferon α therapy, psychotic disorders have been rarely reported.

We present a case of Interferon α2b induced psychotic symptoms in a young male with chronic hepatitis B nine weeks after administration of a standard dose of interferon alpha 2b followed by a review of the literature.

CASE

A 19 year old male, a student by profession, presented to the gastroenterology services of the Aga Khan University Hospital, Karachi, Pakistan with a four months history of lethargy, easy fatigability, decreased appetite and a weight loss of 6 kg. History revealed that he had tested positive on screening for Hepatitis B Surface Antigen (HBsAg). Examination was unremarkable. Hepatitis Be Antigen (HBeAg) was reactive. Alanine aminotransferase (ALT) levels were elevated. Serology markers for hepatitis C and delta virus were negative. Abdominal ultrasound showed a normal sized liver with a coarse echotexture. A normal portal vein and spleen were visualized and no evidence of hepatocellular carcinoma or ascites was detected. His prothrombin time was normal and serum albumin was 4 gm/dl. Liver biopsy was not performed due to reluctance on the patient's part.

He was started on anti-viral therapy that included Interferon α2b (five million units, subcutaneously, daily for four months). Treatment continued for nine weeks without complications apart from a drop in platelet count to110 X 10E9 / L without any signs of bleeding and ALT levels improved.

Nine weeks after initiation of treatment he presented to the psychiatry services, with a two day history of confusion, disorientation and visual hallucinations. He was reported to be talking 'gibberish' and was at times, incoherent. On examination, the patient was conscious but confused and perplexed, had poor concentration and was easily distractible. His approach was aggressive and he was in a delusional state. He was not oriented to time, place or person. According to the informants he had decreased appetite and sleep and was "seeing animals like lions and snakes". Past psychiatric and family history was unremarkable. Pre-morbidly he had been friendly, sociable and hard-working. Nine months into his treatment he had received 90 doses of Interferon α2b and 30 more were due. A provisional diagnosis of a sub-acute delirious episode secondary

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to Interferon α2b therapy was made and he was admitted to the hospital for further evaluation and management.

Even though he was started on a small dose (0.5mg) of Haloperidol (Serenace®), three times a day, extra-pyramidal side effects appeared shortly after initiation of treatment. Haloperidol was discontinued and the patient was started on Risperidone (an atypical antipsychotic) 0.5 mg twice a day. In consultation with his gastroenterologist, Interferon α2b therapy was withheld.

Over the next few days, he became more alert and responsive. His speech improved and became more coherent and he regained his appetite and sleep gradually. Risperidone (0.5mg twice a day) was continued and by the sixth day of admission his symptoms had resolved satisfactorily. He was discharged home with advice to follow-up in the out-patient clinic.

A follow-up visit a week later showed that he had improved clinically. He was alert and attentive and his sleep- wake cycle and appetite had returned to premorbid state. He had little recollection of his symptoms and was keen to resume his studies. Three weeks later investigations showed that his platelet counts and ALT levels were within normal limits. However, his hepatitis Be Antigen remained Reactive. He was advised to gradually decrease and then to stop Risperidone. He was subsequently seen by the gastroenterologist and it was decided to start him on Lamivudine 100mg daily orally for one year. Although his ALT levels had returned to baseline his HBeAg was still reactive and he is expected to need the anti – viral therapy for another two to three years unless he seroconverts or develops resistance to Lamivudine; in that case, he will be switched to another oral anti – viral agent.

DISCUSSION

Psychiatric symptoms related to Interferon α therapy for chronic hepatitis have been a crucial issue in consultation liaison psychiatry. Interferon α induced psychiatric symptoms primarily fall into 3 categories: 1) An organic personality syndrome characterized by irritability and short temper 2) An organic affective syndrome marked by extreme emotional lability, depression and tearfulness; and 3) A delirium-like state marked by clouding of consciousness, agitation, paranoia and suicidality.[7] Amongst psychiatric symptoms related to Interferon α use, depression with irritation and anxiety are the most commonly reported while psychotic symptoms are relatively rare.[8] These side effects usually appear after one to three months of therapy, improve within three to four days of decreasing the dose; invariably resolving once Interferon α therapy is discontinued.[7]

Interferon α induced psychiatric manifestations are widely recognized but the toxicity mechanisms are not clearly understood. The fact that this toxicity appears to be dose-dependent with variation depending on the daily dose given, the mode of administration, combination with other chemotherapy treatments, the concomitance with cerebral radiotherapy or a medical history of psychiatric illness, has been widely accepted.[9] However, few scientific studies have addressed the question of mechanism of Interferon associated neuro-psychiatric changes and yet fewer have come up with convincing explanations.

Though well known that Interferon α affects neuro-endocrine, cytokine and neurotransmitter pathways, it is not clear how that leads to the psychiatric complications.[10,11] Possible mechanisms could involve secondary cytokines and neuro – endocrine systems. Secondary cytokines may activate the hypothalamic pituitary axis, which may in turn cause depressive symptoms or persistent elevation of amines in patients treated with Interferon α providing another possible cause for depression in these cases.[12]

However, psychotic symptoms secondary to Interferon α continue to perplex researchers around the globe. Manipulation of various CNS receptors including dopamine, serotonin, opioid and glutamate receptors might give a clue as to why psychosis arises and address the reason for its rare occurrence.[6]

Review of the literature shows that Interferon α therapy should be discontinued in patients with moderate to severe suicidal ideation or those who have attempted suicide as well as in those with depression that does not respond to anti-depressant treatment in manic states and in individuals with hallucinations, delusions and delirium.[7,13,14]

On the basis of our knowledge of the pharmacological action of Interferon α, several treatment options may be suggested, but these lack empirical support at this time. Antidepressant use for this situation has been reported twice; fluoxetine[15] and nortriptyline[16] were successfully used in two patients treated with Interferon α for hepatitis C and all depressive symptoms and fatigue resolved. Naltrexone (opioid receptor antagonist) has been shown to be beneficial for cognitive impairment.[17]

For other disorders, conventional therapy has been proposed: lithium for bipolar disorders, fluvoxamine for obsessive compulsive disorders and neuroleptics for psychotic disorders.[9] Non – steroidal anti-inflammatory drugs (NSAIDs) hold a promising future for the treatment of psychiatric side effects of Interferon α.[8] Non – pharmacological interventions may also help. Education regarding possible neuropsychiatric changes secondary to Interferon α use,[17] behavioral interventions (such as distraction

and alteration of work and recreation schedules), aerobic exercises[18] and supportive psychotherapy may improve tolerance of symptoms, but currently data on these interventions is lacking.

As the incidence of hepatitis B and C induced chronic hepatitis rises in Pakistan, it is expected that more patients would be treated by Interferon. In this context, early recognition and treatment of neuro-psychiatric side-effects becomes important. This will have implications for treatment compliance as well as adequate control and remission of hepatitis.

CONCLUSION

The appearance of neuro-psychiatric side effects during chemotherapy using the Interferon α molecule is a relatively frequent complication, which at times can have serious consequences, the most important of which is discontinuation of treatment. In many cases this can prove to be fatal. Despite its relatively rare occurrence, psychosis can emerge during Interferon α therapy and its early recognition and treatment is important for a better prognosis. More basic descriptive research is needed in order to adequately design an intervention trial in this regard. This would help to prevent and treat complications and optimize Interferon α therapy in patients suffering from chronic viral hepatitis and malignancies.

NOTES ABOUT CONTRIBUTORS

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REFERENCES

1. Mahoney FJ, Kane M. Hepatitis B vaccine. In: Plotkin SA and Orenstein WA, eds. Vaccines, 3rd ed. Philadelphia: W.B. Saunders Company, 1999:158–182.
2. Robinson WS. Hepatitis B virus and hepatitis D virus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 4th ed. New York, Churchill Livingstone, 1995:1406–1439.
3. World Health Organization; Media Center; Fact Sheets: Hepatitis B; Revised October 2000. Available from: http://www.who.int/mediacentre/factsheets.
4. Ahmad K. Pakistan: a cirrhotic state? Lancet 2004;364:1843-4.
5. Schaefer M, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Schmidt F, et al. Interferon alpha (IFNalpha) and psychiatric syndromes: a review. Prog Neuropsychopharmacol Biol Psychiatry. 2002;26:731-46.
6. Schaefer M, Schwaiger M, Pich M, Lieb K, Heinz A. Neurotransmitter changes by interferon-alpha and therapeutic implications. Pharmacopsychiatry. 2003;36 Suppl 3:S203-6.
7. Renault PF, Hoofnagle JH, Park Y, Mullen KD, Peters M, Jones DB, et al. Psychiatric complications of long-term interferon alfa therapy. Arch Intern Med. 1987;147(9):1577-80.
8. Gallegos-Orozco JF, Fuentes AP, Gerardo Argueta J, Perez-Pruna C, Hinojosa-Becerril C, Sixtos-Alonso MS, et al. Health-related quality of life and depression in patients with chronic hepatitis C. Arch Med Res. 2003;34(2):124-9.
9. Neri S, Pulvirenti D, Bertino G. Psychiatric symptoms induced by antiviral therapy in chronic hepatitis C: comparison between interferon-alpha-2a and interferon-alpha-2b. Clin Drug Investig. 2006;26(11):655-62.
10. Nemeroff CB, Krishnan KR, Reed D, Leder R, Beam C, Dunnick NR. Adrenal gland enlargement in major depression: a computed tomographic study. Arch Gen Psychiatry 1992;49:384–387.
11. Stokes PE, Sikes CR. The hypothalamic – pituitary – adrenocortical axis in major depression. Endocrinol Metab Clin North Am 1988;17:1–19.
12. Miller A. Neuroendocrine and immune system interactions in stress and depression. Psychiatr Clin North Am 1998;21:443–463.
13. Valentine AD, Meyers CA, Kling MA, Richelson E, Hauser P. Mood and cognitive side effects of interferon-alpha therapy.Semin Oncol. 1998 ;25(1 Suppl 1):39-47.
14. Renault PF, Hoofnagle JH. Side effects of alpha interferon. Semin Liver Dis 1989;9:273-277.
15. Levenson JL, Fallon HJ. Fluoxetine treatment of depression caused by interferon alpha. Am J Gastroenterol 1993;88:760–761.
16. Goldman LS. Successful treatment of interferon – alpha induced mood disorder with nortriptyline (letter). Psychosomatics 1994;35:412–413.
17. Dicperink E, Willenbring M, Samuel HO. Neuropsychiatric symptoms associated with Hepatitis C and Interferon Alpha: A review. Am. J Psychiatry 2000;157:867-876.
18. Byrne A, Byrne DG. The effect of exercise on depression, anxiety and other mood states: a review. J Psychosom Res 1993;37:565–574.

EXHIBIT H

Note: Performing your original search, ***psychosis interferon*** , in PubMed will retrieve *103* records.

1: Ann Pharmacother. 2003 Mar;37(3):384-7.

Psychosis associated with interferon alfa therapy for chronic hepatitis B.

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OBJECTIVE: To report a case of persistent psychosis that developed during interferon alfa (IFN-alpha) therapy for chronic hepatitis B. CASE SUMMARY: A 26-year-old man who was diagnosed with active chronic hepatitis B began treatment with IFN-alpha. Five months after initiation of therapy, he developed acute psychosis with prominent persecutory delusions and auditory hallucinations. Despite discontinuation of IFN-alpha therapy and addition of antipsychotic drug treatment, only partial recovery from psychosis was observed after 4 months of hospitalization. DISCUSSION: Unlike many previously reported cases, this patient showed only partial recovery from psychosis, despite the discontinuation of IFN therapy. Except for receiving a relatively high dose of IFN-alpha (10 million units 3 times/wk), the patient did not have any previously proposed risk factors for developing psychiatric adverse effects. The Naranjo probability scale indicates a probable relationship between the acute psychosis and IFN therapy. CONCLUSIONS: Despite its rare occurrence, psychosis can emerge during IFN-alpha therapy. This adverse effect may persist for several months, even after appropriate medical management. IFN-alpha should be used with careful monitoring of patients' psychiatric status during all stages of therapy.

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